EVALUATION OF PROTEIN NUTRITION

L;32=02
N59
4381

CFTRI-MYSORE
4381
Evaluation of pr

C.F.T.R.I.

4381

Proteins, ~~Amino acides~~,
Amino acid requirements.
Protein requirements,
Protein deficiency,
Amino acid "
Amino acids, Proteins, Diet,
Amino acid supplementation,
Nutritional evaluation,

Evaluation of Protein Nutrition

A Report of the
Food and Nutrition Board

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
LIBRARY
MYSORE.

573
E9

National Academy of Sciences—
National Research Council

Publication 711

C. F. T. R. I. LIBRARY, MYSORE

Acc. No. 4381

Call No. L;32=02. N59

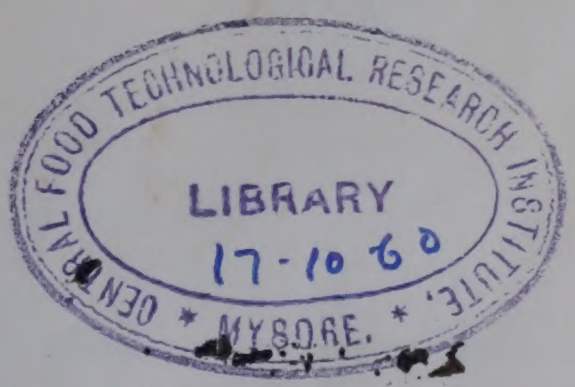
Please return this publication on or before the last DUE DATE stamped below to avoid incurring overdue charges.

P. No.	Due date	Return date
264	22/6/62	21/6
373.	21/7	31/7/62
215.	11/10	11/10
305.	11/11	10/10
318	15/11/62.	14/11
380	15/5/63.	14/5/63
43	14/6/60	12/6/63.
121	24/6/63.	24/6
169.	12/7.	15/7/63
241.	30/7/63	28/7/63
157	12/10/63	11/10/63

Evaluation of Protein Nutrition

A Report of the
Food and Nutrition Board
Division of Biology and Agriculture

Prepared by the
Committee on Amino Acids



Publication 711
NATIONAL ACADEMY OF SCIENCES—NATIONAL RESEARCH COUNCIL
Washington, D. C.
1959

Price $2.00

4381
G. 809
L; 32 = 02
N59

CFTRI-MYSORE
4381
Evaluation of pr..

Contents

	PAGE
Foreword	v
Preface	vii
Basic Concepts	1
Metabolism of Protein and Amino Acids	1
Nitrogen Balance as Used in Study of Protein Nutrition	2
Nutritive Value of Dietary Proteins	3
Minimum Nitrogen Excretion	6
Amino Acid and Protein Requirements	9
Amino Acid Requirements of Animals	9
Amino Acid Requirements of Humans	9
Amino Acid Proportionality Patterns	13
The Protein Requirement	15
Effects of Deficiency of Proteins and Amino Acids	19
Effects of Protein Deficiency in Experimental Animals	19
Skeleton	20
Connective tissue cells	20
Reproductive tract	20
Endocrines	20
Skin	21
Eye	21
Pancreas	21
Kidney	21
Nervous tissues	21
Muscle	21
Bone marrow and blood cells	21
Liver	21
Immune responses	21
Effects of Amino Acid Deficiency in Experimental Animals	22
Tryptophan	22
Lysine	22
Isoleucine	22
Threonine	22
Methionine	23
Valine	23
Syndromes Associated with Protein Depletion in the Human	23
Hunger edema	23
Pellagra	24
Kwashiorkor	24
Nutritional liver disease	25

PAGE

Effects of Disproportionate Amounts of Amino Acids 27
Effects of Administering Excessive Amounts of Individual Amino Acids . 28
Toxic effects 28
Parenteral administration 28
Oral administration 28
Amino Acid Antagonism 30
Amino Acid-Vitamin Relationships 30
Amino Acid Balance and Imbalance 31
Amino acid balance 31
Amino acid imbalance 32
Growth 32
Liver fat deposition 32
Liver enzymes 33
Protein and Amino Acid Content of Diets in the United States 35
Protein in U. S. Food Supply 35
Protein in Family Diets 35
Protein in Diets of Individuals 36
Amino Acids in U. S. Food Supply 37
Amino Acids in Diets of Individuals 38
Amino Acid Patterns 39
Amino Acids in Meals 40
Appraisal of Protein Adequacy 41
Problems of Improving Protein Nutrition by Amino Acid Supplementation . . 43
Experimental Background 43
Rationalization for Adding Lysine to Cereal Products 44
Problem of Amino Acid Balance 45
Relation to General Policy of Food Fortification 47
Summary and Evaluation 49
Figure 1—Idealized Curves Illustrating Effect on Nitrogen Balance of Four Proteins Having Different Nutritive Value 3

Tables

		PAGE
1.	Average Minimal Daily Amino Acid Requirements of Humans	10
2.	Calculated Amino Acid Intakes (mg/kg) of Infants on Natural Diets Compared with Requirements Directly Determined	11
3.	Calculated Amino Acid Intakes of Adults (g/day) from Experimental Diets as Compared with Minimal Requirements	12
4.	Proportionality Patterns of Amino Acid Requirements of Humans	13
5.	Amino Acid Proportionality Pattern for the Adult and Its Comparison with Egg and Milk Proteins	14
6.	Calculated Minimal Protein Requirements of Humans as Grams per Kilogram of Body Weight	16
7.	Sources of Protein in U. S. Food Supply	35
8.	Distribution of Households by Protein Content of Food Supplies per Nutrition Unit per Day	36
9.	Amino Acids in U. S. Food Supply and a Comparison with Adult Requirement Pattern	38
10.	Homemakers' Diets: Calories, Niacin and Amino Acid Content of Selected One-Day Diets Providing 40 gm of Protein	39
11.	Amino Acid Patterns in One-Day Diets of Individuals, Compared with Specified Patterns (Tryptophan = 1.0)	40

Foreword

The initial assignment presented to the Committee on Amino Acids was that of evaluating the merits of the addition of specific amino acids to processed food as a means of enhancing the nutritive value of dietary protein. The Committee was specifically instructed to keep in mind the *Statement of General Policy in Regard to the Addition of Specific Nutrients to Foods,* adopted jointly by the Food and Nutrition Board and the Council on Foods and Nutrition of the American Medical Association in November 1953.

Early in its deliberations the Committee concluded it could not judge the potentialities of amino acid supplementation without a critical consideration of the current knowledge of protein and amino acid requirements in relation to current dietary protein and amino acid consumption. In order to expedite compilation of the latter information, the Committee issued a public plea in February 1953 for data to be furnished the United States Department of Agriculture to help in the compilation, subsequently published by USDA as *Amino Acid Content of Foods.*

In 1957 the Food and Nutrition Board adopted and published a precautionary statement developed by the Committee on the *Supplementation of Dietary Proteins with Amino Acids.*

The Committee, because of the world-wide interest in protein nutrition, has not confined its evaluation to the United States of America, and has taken full advantage of information developed through the United Nations Agencies and through the Board's Committee on Protein Malnutrition.

The Food and Nutrition Board is grateful for the intensive work of the Committee on Amino Acids in the preparation of this report.

C. A. ELVEHJEM, *Chairman,*
Food and Nutrition Board, 1955-1958.
G. A. GOLDSMITH, *Chairman,*
Food and Nutrition Board, 1958- .

Preface

The Committee on Amino Acids of the Food and Nutrition Board, Division of Biology and Agriculture, National Academy of Sciences—National Research Council, has attempted to survey and evaluate the status of knowledge concerning dietary protein needs. There is a lack of precise knowledge concerning protein requirements, especially in children in various environments. Even in the United States and other countries where dietary proteins are relatively abundant, there is a need to define further the optimal intake for desirable rate of growth and development, and for the maintenance and regeneration of tissue proteins. The role, desirable level, and means of maintenance of the so-called "protein reserves," the role of protein in the development of resistance to and recovery from the various stresses, and the effect of protein on longevity and development of the so-called degenerative diseases are problems that require further investigation. More emphasis needs to be focused on the study of the quality of a protein *vis-a-vis* the quantity. If the needs can be demonstrated, both the quality and quantity of protein in many basic foods can be increased by supplementation with either protein or amino acids. Finally, the effects of excessive protein and amino acid intake need further investigation since the problem of dietary surfeits may prove to be important to the health and welfare of a population.

The Committee On Amino Acids

J. B. Allison, *Chairman*

J. S. Andrews
R. H. Follis, Jr.
A. E. Harper
D. M. Hegsted
L. E. Holt, Jr.
Esther F. Phipard
H. H. Williams

Basic Concepts

Some twenty-two or more different amino acids are needed for the growth of new tissues, for the maintenance of established cellular constituents, and for other metabolic requirements. The function of a dietary protein is to supply the nitrogen for the synthesis of a variety of nitrogen-containing compounds (heme, creatine, hexosamine, dispensable amino acids) and to provide those amino acids that cannot be synthesized in adequate amounts. The latter are called "essential" or "indispensable" since they must be supplied by the diet in proper proportions and amounts to meet the requirements for maintenance and growth of tissue and for other metabolic processes (180). The amino acids which can be synthesized in sufficient quantities for the needs of the body are called "non-essential," or "dispensable." Although the amino acids which are indispensable for various species show general similarity, there are some species differences. Also the rates of synthesis and relative need for various amino acids vary somewhat with the physiological state. Thus certain amino acids may be dietary essentials under some conditions but not under others.

Metabolism of Protein and Amino Acids

Amino acids enter into the general metabolic pathways which have been called anabolism and catabolism. Anabolism is the process of synthesis, whereby, for example, amino acids are organized into cellular proteins. Catabolism is the reverse process, resulting in the hydrolysis of the proteins to amino acids, and the breakdown of the amino acids into simpler products. The breakdown of proteins contributes amino acids to a so-called "metabolic pool." Thus, the tissue proteins to varying degrees are in a dynamic state, contributing amino acids to the "pool" and taking amino acids from it. A portion of the amino acids from either food or body protein enters into energy catabolism leading to the formation and excretion of urea. Other nitrogenous compounds such as ammonia, creatinine, uric acid, and small amounts of amino acids are also excreted. Folin (77) suggested that compounds such as creatinine which are derived from the metabolism of muscle cells and are formed at a rate essentially independent of dietary nitrogen intake should be considered as endogenous in origin. Similarly, the urea nitrogen which results from the catabolism of tissue protein can be considered as endogenous in origin, but that portion of urea which is derived from the catabolism of dietary amino acids would be called exogenous. Obviously, it is difficult to separate nitrogen losses into those of body and of food origin when the nitrogen comes from a common "metabolic pool" contributed to by both tissue and dietary proteins. Nevertheless, the concept of endogenous (body) and exogenous (food) metabolism has been a valuable contribution to the determination of nutritive value of dietary proteins (155) and will be considered later in this report.

Early in the study of the synthesis of plasma proteins, Whipple and associates (243) emphasized the possibility of a dynamic state among various protein compartments of the body, a state that could lead to an "ebb and flow" of nitrogen from one compartment to another. This dynamic state was given more consideration through the studies of Schoenheimer, Rittenberg, and associates (177) who revealed an interchange of isotopic nitrogen within the body proteins. Different tissue proteins, however, vary in their "half lives." Some proteins, for example collagen, may be considered to be

"one-way streets," taking from but contributing little to a common "metabolic pool." Some tissue proteins with relatively short "half lives" decrease rapidly with a reduction in quantity or quality of dietary protein. Others may be in a highly dynamic state, but breakdown and resynthesis may be so balanced that they appear relatively constant in concentration and independent of dietary protein intake. Those tissue proteins that rise and fall with changes in dietary protein intake are sometimes called the "labile protein reserves" of the body, since they are depleted or repleted as the protein content of the diet is varied (13). Various proteins of the liver and other tissues (2) represent a portion of the reserves. Enzyme systems such as amino acid oxidase, succinic acid dehydrogenase, xanthine oxidase, serum aldolase, plasma pseudocholinesterase, and many others decrease in activity as the tissue proteins are depleted (152, 238, 239, 250). Some changes in the activity of enzyme systems and organ size (2) represent adaptation, independent of the magnitude of the "protein reserves." Thus a protein-depleted individual may be considered to be depleted in various protein compartments such as plasma proteins, tissue proteins, and a "metabolic pool" contributed to by all the soft tissues of the body. One of the major problems in nutrition is to determine what constitutes the optimum development of these protein compartments.

Nitrogen Balance as Used in the Study of Protein Nutrition

Since nitrogen is continuously being lost from the body, it is possible to strike a balance between nitrogen intake and nitrogen excretion as follows:

$$B = I - (U + F) \qquad (a)$$

where B is nitrogen balance, I is nitrogen intake, U is urinary nitrogen excreted and F is fecal nitrogen (15). Nitrogen is also lost from the sweat glands in man, a loss which is very difficult to estimate and most often neglected. It is possible that under certain conditions this neglect may result in significant misinterpretations of the data. If the intake I is greater than the total nitrogen excreted, then the balance B is positive and the individual is gaining in body nitrogen. If I equals excretion, the individual is said to be in nitrogen equilibrium. If I is less than (U+F), then the balance B is negative and the body is losing nitrogen. Nitrogen balance is the net result of gains and losses in all the protein compartments of the body and, therefore, gives no indication of shifts in the distribution of nitrogen among the various compartments.

Some information on the dietary protein requirements can be obtained from the amount of nitrogen intake necessary to establish equilibrium (nitrogen balance equal to zero). Interpretation is, however, subject to certain limitations which must be appreciated, because over a period of time the body adapts to an altered nitrogen intake. An increased intake is followed by an increased turnover of nitrogen and increased excretion in the urine, and the converse will occur when the intake is decreased. In either event, nitrogen equilibrium is reestablished at the new level of intake. For example, an adult dog, adapted to a low protein intake, may establish nitrogen equilibrium at an intake of 0.1 gm of nitrogen per day per kg of body weight, while a dog, previously adapted to a higher protein diet may require 0.25 gm of nitrogen per day per kg of body weight to do so. On the low intake, adaptation is accompanied by reduction in tissue proteins, possibly some of the less essential decreasing more rapidly and the more essential being spared according to a system of priorities. Undoubtedly the individual adapted to a low nitrogen intake is more vulnerable to certain stresses and to that extent may be said to lack "protein reserves." On the other hand, the individual adapted to what may be excessive nitrogen intake, as opposed to an optimum protein diet, may develop hypertrophy of tissues, particularly of those (liver, kidney, adrenal) concerned in handling metabolic excesses. Requirements for nitrogen equilibrium, therefore, cannot alone provide an estimate of the amount of protein needed for optimum

performance and some other method of estimating a "desirable" state of "protein reserve" is needed. Unfortunately this is not readily available.

In growing animals the amount of tissue formed can be estimated by the degree of positive balance. The amount of nitrogen retained will depend in part upon the previous diet of the animal, the amount of protein fed during the study, and the rate of growth of the animal. Since maximum retention of nitrogen or maximum growth cannot necessarily be equated with optimum health, the difficulties of estimating requirements during growth and in the adult are essentially similar although the criteria are somewhat different.

Nutritive Value of Dietary Proteins

The ability of dietary proteins to meet nitrogen requirements varies, a variation that is an expression of nutritive value (13). The nutritive value of proteins in meeting nitrogen requirements must be considered in relation to the utilization of proteins for energy.

Differences in nutritive value of dietary proteins can be illustrated by the effect of increasing nitrogen intake upon nitrogen retention in the body as measured by nitrogen balance. The rate of change of nitrogen balance with nitrogen intake is illustrated in figure 1 by idealized curves. Such data are obtained from short-term studies in which the organism is not allowed time to adapt to

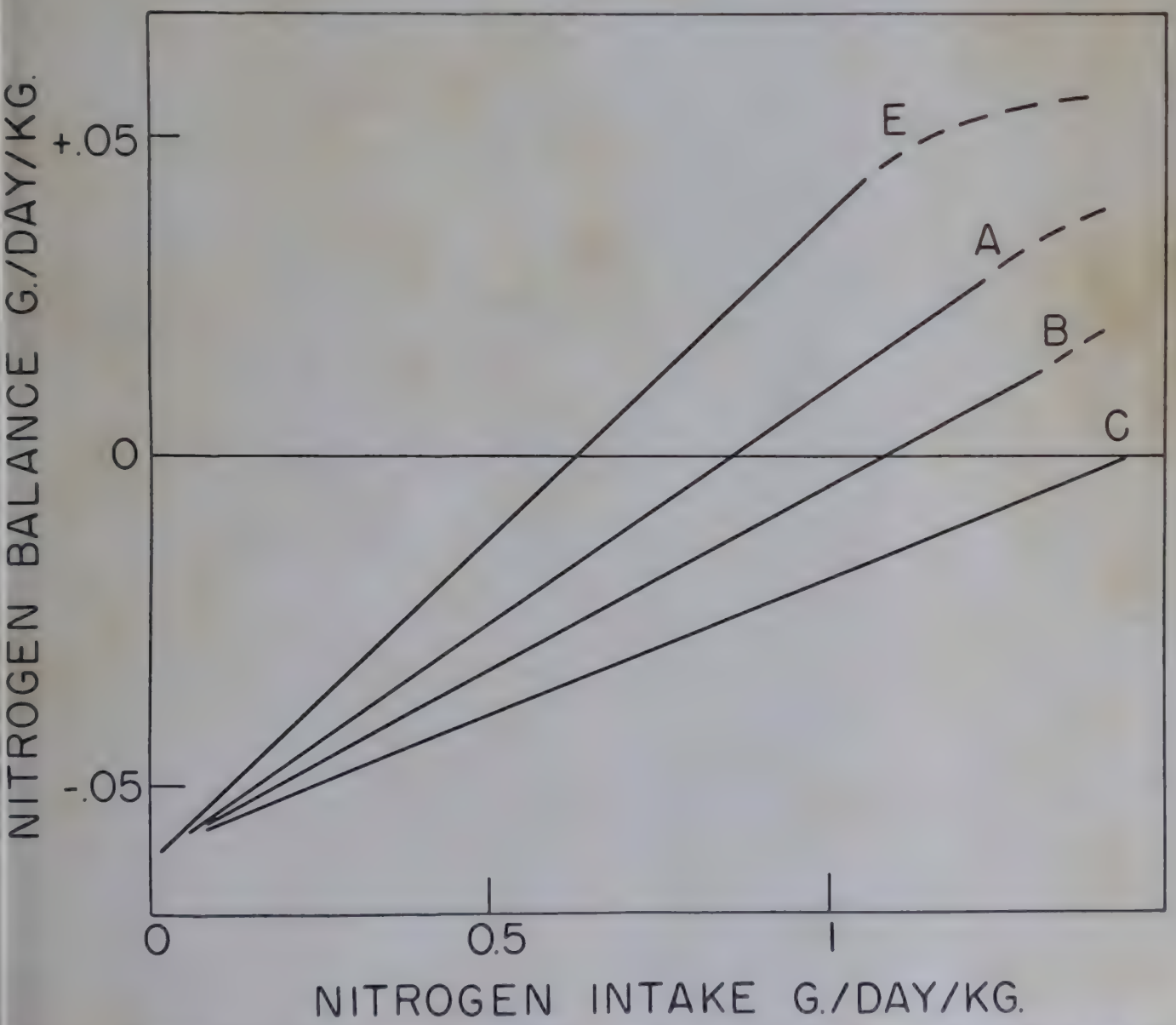


Figure 1. Idealized curves illustrating effect on nitrogen balance of four proteins (A, B, C and E) having different nutritive value.

the change of diet. Curve E records the type of data obtained while feeding different amounts of egg protein nitrogen for short periods of time. The curves A, B, and C illustrate the type of data obtained by feeding proteins of lesser nutritive value (15, 109). The slopes of these lines have been called the nitrogen balance indexes of dietary protein; the greater the slope, the more efficiently the protein is utilized for maintenance and growth (13). The amount of nitrogen needed for maintenance is recorded in figure 1, where the nitrogen balance is zero. Positive balance signifies either the growth of new tissues or the filling of protein reserves in depleted tissues already formed (16). If the "protein reserves" are depleted, the body may respond by reducing the excretion of nitrogen and requiring less nitrogen for maintenance. Under these conditions of depletion, by increasing the nitrogen intake an individual will go into positive balance. Conversely, if the nitrogen intake of an individual on a high nitrogen intake is reduced, he will go into negative balance. In either event eventually nitrogen equilibrium is achieved at the different intake (15).

The curves in figure 1 are usually considered to be linear in the region of negative balance, the linearity extending into positive balance if there is a potential for growth of new tissue or repletion of old, but eventually the lines become curvilinear in the region of positive balance. If the animal is an adult with no potential for growth and if the "protein reserves" are filled, then the line could not extend into positive balance. Experimentally, the curves are not always found to be linear (160) but they always express the rate of retention of nitrogen with respect to intake. For that reason the tangent to the curve at any intake has been called the nitrogen balance index of that intake (13). The curves also could be considered essentially semilogarithmic over a wide range of intakes (20, 76). Indeed many biological responses plotted against the variable responsible for that response results in a semilogarithmic relationship expressing the "law of diminishing returns." Thus, to fill the protein reserves most rapidly the intake producing the maximum nitrogen balance would be fed.

By assuming a constant rate of catabolism of body tissue proteins, the nitrogen balance index (nitrogen retained) in per cent can be calculated from the following equation:

$$\text{Nitrogen Balance Index} = \frac{100(B - B_0)}{I} \qquad (b)$$

where B is the nitrogen balance produced by the nitrogen intake I, and B_0 is the unavoidable excretion of body nitrogen (given a negative sign) and is considered to be constant and independent of nitrogen intake if this equation is to represent true nitrogen retention. The value for B_0 is usually estimated by measuring the nitrogen balance of an individual fed a protein-free diet. The nitrogen balance of a man fed a protein-free diet was, for example, -0.056 gm/day/kg body weight. Egg protein, equivalent to 0.065gm N/day/kg, was fed and the nitrogen balance produced was slightly on the positive side equal to 0.005 gm. Substituting these values in the above equation, the nitrogen retained was 100 $(0.005+0.056)$ divided by 0.065, or 93 per cent. Similarly, 0.14 gm of C protein nitrogen (see figure 1) was needed to reach a nitrogen balance equal to zero. The per cent nitrogen retention was 100 $(0+0.056)$ divided by 0.14, or 40. It should be emphasized that this equation assumes a linear relationship between nitrogen intake and the nitrogen balance produced, but the relationship becomes curvilinear eventually in the region of positive balance. Thus for equation (b) to hold, B should be near or below equilibrium in the adult, or in the region of linearity between nitrogen intake and balance in the growing individual or in the repleting adult (13). The percentage retention of nitrogen, calculated from equation b, is a measure of the overall nutritive value of the dietary protein since it includes the nitrogen lost from the body through the feces as well as through the urine. The nitrogen balance index multiplied by the fraction of dietary protein in the foodstuff is called the net protein value (NPV) of the foodstuff (156). This value is a function of re-

tention of nitrogen by the animal, digestibility, and the concentration of protein in the source.

The nitrogen lost in the feces is generally assumed to be composed of unabsorbed food nitrogen and nitrogen lost from the body into the gastro-intestinal tract (metabolic nitrogen or fecal nitrogen of endogenous origin). In order to estimate the amount of food nitrogen in the feces, the simplest method is to assume that the food fecal nitrogen can be calculated according to equation c:

$$\text{Fecal food nitrogen} = (F - F_0) \qquad (c)$$

where F is total fecal nitrogen and F_0 is fecal nitrogen of endogenous origin, measured while feeding a protein-free diet. Using this assumption, absorbed nitrogen A is calculated as follows:

$$A = I - (F - F_0) \qquad (d)$$

and digestion (D) (actually absorption) would be

$$D = \frac{A}{I}. \qquad (e)$$

It should be clear that such a method assumes that F_0 is a constant, an assumption which is not always entirely valid (41).

The Biological Value of a protein, a concept originated by Thomas (231) and developed so well by Mitchell (155), is defined as the percentage of absorbed nitrogen retained in the body. The term Biological Value is often used loosely as synonymous with nutritive quality but it seems preferable to use this term in the strict sense as nitrogen which is retained. If it is assumed that B_0 in equation (b) is the excretion of body nitrogen and is independent of nitrogen intake, then

$$\text{Biological Value} = \frac{100(B - B_0)}{A} \qquad (f)$$

if B is near nitrogen equilibrium in the adult, or in the region of positive balance where the relationship continues to be linear between absorbed nitrogen and positive nitrogen balance in the growing young or replet-ing adult. Equation (b) and the Biological Value calculated from equation (f) would be identical if digestion was complete so that I was equal to A. The net protein value (NPV) of a foodstuff would be obtained by multiplying D (in equation (e)) by the Biological Value and by the fraction of dietary protein in the foodstuff. The Biological Value for egg proteins is usually close to 100, which can be interpreted to mean that all of the absorbed nitrogen is retained in the body. Actually it demonstrates that the dietary nitrogen from egg protein is able to replace gram for gram the losses which unavoidably occur. These results can be interpreted to mean that the amino acids supplied by whole egg are in the proper proportion to provide maximum efficiency of utilization.

The Biological Values of numerous proteins have been measured, utilizing primarily the rat as the test organism. Fewer data are available upon other species, including human subjects. However, since the amino acid composition of the body proteins of different species is quite similar, it might be expected that the Biological Value of a protein would be similar for the different species (155). Insofar as data are available, this appears to be essentially true. The exceptions occur, as would be expected, primarily when large amounts of proteins of unusual composition must be formed, as in wool and feathers. The chick also lacks ability to synthesize arginine and has only limited ability to synthesize glycine. The present evidence indicates that adult man but not the infant differs from other species in not requiring histidine (179). The Biological Values of proteins for the rat, dog, pig, and man appear to be similar and values determined with the use of these species are probably generally applicable to all.

If, as indicated, Biological Value is primarily an estimate of how closely the amino acid composition of the protein conforms to a pattern of amino acids utilized most efficiently by the body, it should be possible to estimate Biological Value from a knowledge of the amino acid composition. Various attempts have been made to demonstrate this (130, 167). The best known is the report of Block and Mitchell (37, 157). Using egg protein with a Biological Value of 100

as an appropriate standard, these authors compared the percentage of each indispensable amino acid to that in egg protein. The relative amount of amino acid showing the largest deficit as compared with egg was taken as the "protein score," since this should be the amino acid limiting the utilization of the total protein. This score was rather highly correlated ($r=0.83$) with the experimentally determined Biological Value (rats).

However, Mitchell (156) has since shown that a considerable improvement in this correlation is achieved by modifying the method used by Oser (167) to arrive at the score. The "modified amino acid index" is the geometric mean of the relative deficits of all of the indispensable amino acids present in smaller quantities than occur in egg protein. This correlation, considering the errors inherent in both the Biological Values and the amino acid composition figures, is surprisingly high. It suggests that the Biological Value is determined, essentially, only by the amino acid composition. The improvement in correlation obtained by this index, as compared with the original method of Mitchell and Block, indicates that the single amino acid most limiting does not entirely determine the Biological Value but that all deficits contribute. Recent studies (117), however, reemphasize the value of the simpler approach to an estimation of nutritive value through the single most limiting amino acid.

Possibly the best reference pattern for estimating the nutritive value of dietary proteins will eventually be developed from studies of amino acid requirements for maintenance, growth, and other metabolic needs (14). It was with this possibility in mind that the FAO Committee on Protein Requirements (80) devised a reference pattern from data available on the amino acid requirements of man (5-12, 115, 116, 133-137, 150, 178-191, 213-217, 227-228).

These data are so limited that such a reference pattern must be considered as a first step in that direction. Some of the proportions of essential amino acids represented in a reference pattern derived from amino acid experiments (19) are relatively lower than those found in egg, a difference which may result from experimental errors in the determination of the amino acid requirements. It is possible also that, in order to meet the nitrogen requirements while feeding egg protein, an excess of some of the essentials is provided to the body, an excess that then contributes to the overall basic need for nitrogen. A comparison of dietary proteins with either the FAO (80) or egg reference patterns suggests that the limiting amino acids generally are lysine, tryptophan, and methionine.

Minimum Nitrogen Excretion

As has been indicated previously, nitrogen balance measurements have limitations as estimates of protein requirements. Possibly there is one parameter at the present time that can be estimated with considerable accuracy. This parameter is the minimum protein requirement per calorie of basal metabolism. Terroine (230) and Sorg-Matter (218) proposed, from comparative studies upon various species, that the minimum nitrogen excretion was proportional to basal metabolism and was equal to about 2 mg of nitrogen per basal calorie. Thus, when an animal is fed a protein-free diet or a diet containing a small amount of egg protein, the urinary nitrogen excretion falls rapidly at first and approaches asymptotically a relatively constant and characteristic figure proportional to the basal metabolic rate. Extensive confirmation of the conclusions of Terroine is available (110, 154, 196, 212). The minimum excretion obtained is as low as 1.4 to 1.5 mg of nitrogen per basal calorie. Values of this order of magnitude were obtained by Bricker, Mitchell, and Kinsman (42) in human subjects. Such evidence as is available suggests that the minimum nitrogen excretion of young animals is of the same order of magnitude per basal calorie. Thus the maintenance requirement of the young animal, apparently as distinct from the growth requirement, appears to be governed by the same laws as are operative in adult animals.

It has been pointed out that when a protein with a Biological Value of 100 is fed

at a relatively low level the urinary nitrogen excretion is not increased, and that the dietary protein replaces, on an equivalent basis, the losses in the urine. Hence, the determination of the urinary nitrogen excretion when the individual receives a nitrogen-free diet is a measure of the amount of nitrogen from a protein of Biological Value 100 which must be absorbed to achieve nitrogen balance. It is an estimate of the protein requirements for equilibrium of the individual at the time. Since Biological Value measures the efficiency with which absorbed nitrogen from various proteins meets the body needs, the amount of absorbed nitrogen required when proteins of lesser Biological Value are fed can be estimated by multiplying the urinary nitrogen by $\frac{100}{BV}$. For example, if the urinary nitrogen excretion of an individual on a nitrogen-free diet was 4 gm, 25 gm (6.25×4) of a dietary protein with a Biological Value of 100 would be required for equilibrium. If the digestibility of this protein was 0.9 (see equation c) then $\frac{25}{0.9}$ or 27.8 gm should be included in the diet. On the other hand, the protein source as fed may contain only 50 per cent protein. Thus $\frac{27.8}{0.5}$ or 55.6 gm of the protein source would be needed to establish balance. If the protein fed had a Biological Value of 70, approximately 36 gm would be required ($25 \times \frac{100}{70} = 35.7$) for balance. Again assuming digestibility to be 0.9, the amount of protein needed for equilibrium would be $\frac{35.7}{.9}$ or approximately 40 gm. If the protein source contained 50 per cent of this protein, 80 gm of the source should be fed.

Emphasis has been placed upon nitrogen balance as a method of determining the amount of dietary nitrogen retained in the body of the animal and thereby the nitrogen and amino acid requirements for maintenance and growth. A survey of basic concepts such as this, however, would be remiss if it did not mention other methods for estimating nutritive value. An excellent method for expressing the net utilization of proteins has been developed recently by Miller and Bender (153) and Bender (30). In this method the value of the protein is determined by the increase in body nitrogen. One of the simplest methods was proposed a number of years ago by Osborne, Mendel, and Ferry (166). They defined the Protein Efficiency Ratio of a dietary protein as the weight gain per gram of protein or nitrogen consumed, with the stipulation that the nutritive value be expressed in terms of the maximum ratio. Hegsted and Worcester (111) found that, under carefully controlled experimental conditions, the weight gain in rats was so highly correlated with the ratio that weight gain alone was sufficient to define nutritive value. It is obvious, however, that weight gain, particularly in children, could be markedly affected by variation in fat stores and shifts in body composition associated with unbalanced diets. Nutritive values of dietary proteins have been estimated also by rate of repletion of protein-depleted adult tissues (85) and by the effect of diet upon specific protein compartments in the body such as plasma proteins (243), liver proteins (129), enzymatic activities (151, 238, 239, 250), etc. Such estimates of nutritive value may reveal specific influences of different patterns of dietary amino acids upon tissue proteins, influences that could not be uncovered through nitrogen balance measurements alone. A nitrogen intake or a pattern of amino acids of high nutritive value for the normal individual may not represent the best intake or pattern of amino acids for various therapeutic measures in the abnormal or ill individual.

Estimates of requirements for essential amino acids and total nitrogen are all based on the assumption that the individual is receiving a balanced diet. The mineral content, the vitamin content, and above all the caloric intake will influence nitrogen retention in the body. Adequate food energy intake conserves body nitrogen and protein

is utilized for calories when energy intake is deficient. Indeed, the growth of new cellular proteins or the repletion of old must be described in three dimensions: the nutritive value of the dietary protein, the nitrogen intake, and the caloric intake (17). It is apparent, of course, that the requirement for anyone constituent in the diet is a function of all the others, so that the aim of nutritionists is to discover and to construct ideally balanced diets. The amino acid requirements, possibly more than any other requirements in the diet, emphasize the importance of this balance.

Amino Acid and Protein Requirements

The primary function of dietary protein for monogastric animals is to furnish indispensable amino acids as demonstrated by the classic experiments of W. C. Rose and associates (179, 180) and to supply additional nitrogen in a form suitable for the synthesis of other nitrogenous components of tissues with the exception of certain vitamins of the B-complex. Numerous studies summarized by Mitchell (156) demonstrate the high correlation between the nutritive value of proteins and their amino acid composition. There appears to be no reason to doubt that the nutritive value of protein depends primarily on the content of the indispensable amino acids although the pattern of the dispensable amino acids is not altogether without significance (92, 174).

Amino Acid Requirements of Animals

To the extent that studies have been made, all animal species except ruminants under all conditions require a continuous dietary supply of isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (6, 38, 149, 178-191). Histidine is required for growth, and for maintenance in the adult rat and dog, but is not needed for maintenance by the adult human (6, 158, 179, 180). Thus, with the exception of adult humans, it is implied that animal tissues lack the ability to synthesize the nine amino acids listed above. Unequivocal confirmation of the latter has been supplied by the studies of Steele (222). Utilizing the radio-carbon technique, he showed that carbohydrate was unable to supply any carbon to the nine dietary indispensable amino acids, except to the methyl group of methionine. In contrast, carbohydrate readily furnished the carbon for synthesis of the dispensable amino acids.

Consequently, on the basis of present evidence, humans require a food source of eight amino acids for maintenance: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. In addition, other animal species require histidine. Arginine, which is an indispensable amino acid for birds, can be synthesized by other species, but since the rate of synthesis may be limited a dietary supply may be necessary for maximum growth (164).

The quantitative amino acid requirements for several animal species have been studied extensively. The values that have been obtained for the rat, the chick, and the pig appear to be on a firmer basis (249) than any which have been so far derived for humans. The reasons for this are undoubtedly obvious to anyone having experience with the use of humans as experimental subjects. Furthermore, many of the various methods (38, 191) applied to the study of experimental animals are unsuitable or impracticable for investigators with human subjects.

Amino Acid Requirements of Humans

The studies that have been made to determine the precise quantitative amino acid requirements of human subjects are summarized in table 1. They are limited to infants under one year of age and to young adults. The procedures utilized in all of these studies involve the nitrogen balance in the adult and, in addition, growth in the infant. The variations possible in the application of this technique, which involve primarily the formulation of an experimental diet, together with its advantages and disadvantages, are reviewed in a recent report by Pratt *et al.* (171). Besides the problems in designing a satisfactory test diet

as noted above, it is evident in reviewing the studies that the several groups of workers differed in their interpretation of what constitutes nitrogen equilibrium. This is especially notable with respect to the studies of female and male adults. The daily minimum requirements as reported and listed in table 1 are not strictly comparable. For the minimum requirements for adult women the criterion is defined as: "The amount of an amino acid which will keep a subject in a zone of nitrogen equilibrium, where the difference between intake and excretion is within ±5 per cent" (133). For adult men, the minimum requirement of an amino acid is: "the smallest amount capable of inducing distinctly positive balance" (182). For the infants the requirement is dependent on the amount of amino acid needed to produce a normal nitrogen retention and weight gain (49).

Further consideration of the infant requirements is probably unwarranted at this time because of lack of comparative studies. All of the studies were done by the same group. However, it should be noted that the values for the requirements of methionine and isoleucine were determined from intakes on the minimal quantities of milk protein that would permit normal growth. In contrast, the values for the remaining amino acids listed in table 1 under infants were obtained using diets in which the nitrogen components were supplied by L-amino acids in the proportions found in breast milk, including the nonessential amino acids (49).

Examination of the minimal amino acid requirements of adults as listed in table 1 points to the obviously lower values for females than males, with the exception of the aromatic amino acids. The explanation

Table 1

The Average Minimal Daily Amino Acid Requirements of Humans

	Infants		Adults					
			Female			Male		
	mg/kg	Ref	mg/Day [1]	Ref		mg/Day [1]	Ref
Histidine	32	*213*					
Isoleucine	90 [2]	*11*	450	*228*		700	*186*
Leucine	150	*217*	620	*137*		1100	*186*
Lysine	105	*215*	500	*124*		800 [3]	*184*
Methionine	85,[2] 65 [4]	*12*	350	*227*		1100, 200 [4]	*185*
Cystine			200	*227*		absent, 810 [4]	*187*
Total Sulfur A.A.			550	*227*		1100, 1010	*187*
Phenylalanine	90	*214*	220	*136*		1100, 300 [5]	*183*
Tyrosine			900	*136*		absent, 1100 [5]	*188*
Total Aromatic A.A.			1120	*136*		1100, 1400	*188*
Threonine	60	*171*	305	*133*		500	*185*
Tryptophan	22	*10*	157	*135*		250	*182*
Valine	93	*216*	650	*134*		800	*189*

[1] The amino acid requirements for adults are expressed as mg/day because of poor correlation with weight of the individual.

[2] Observations based on only 2 subjects, using chemically degraded diets.

[3] A recent report (48) indicates that 2 male subjects receiving a mixed diet supplemented with lysine and furnishing a total nitrogen intake of 9.0 gm per day required between 800-900 mg of lysine daily for positive nitrogen balance.

[4] With cystine present at 50 mg/kg for infants or at 810 mg/day for male adults, the methionine requirement was 65 mg/kg for infants and 200 mg/day for male adults.

[5] With tyrosine present at 1100 mg/day, the phenylalanine requirement was 300 mg/day for male adults.

for this difference is not completely apparent. As noted by Leverton (133) the studies with females differ from those with males as follows: ". . . in the source of dietary nitrogen, the amino acid, mineral, and vitamin intake, the age as well as the sex of the subjects, and probably in many other aspects such as the experimental levels of the . . . (amino acid) . . . fed, the length of period of study and the number of subjects."

It is certainly possible that all of these factors may be operating to bring about the differences observed. To this committee several appear to be more pertinent than others. For example, the criterion for nitrogen equilibrium as noted earlier would favor higher values for males. Possibly more important is the fact that the values for females represent more closely an average of the responses of the subjects, whereas those for the males represent the highest value required to bring about a positive nitrogen balance in a single subject. The remainder of the male subjects studied in any one experiment required a lesser amount of a specific amino acid. Consequently, the values for the males are weighted toward a higher value than is the case for the females. Furthermore, in the studies with males, racemic mixtures of the amino acids were used in the diet in contrast to the use of L-amino acids in the diets of the females. Until the above differences are investigated it remains doubtful that the other factors mentioned previously, such as age, sex, etc. are operating in these particular experiments to yield such obviously different minimal amino acid requirements between male and female adults.

More pertinent to amino acids requirements of humans, however, would seem to be the question—how meaningful are the adult values listed in table 1 for practical nutrition application? In answering this question, one may recall the histidine controversy (49) and recognize that the minimal values are based on maintenance of equilibrium for a period of only 4-10 days. Rose (189) has noted this question with respect to the other essential amino acids in advising "safe" intakes which are double the minimum value listed in table 1. A much greater caloric intake is required to obtain nitrogen equilibrium with diets containing free amino acids, in contrast to diets containing protein. As stated by Rose (181): "The data appear to demonstrate conclusively that diets containing amino acid mixtures . . . necessitate higher caloric intakes for nitrogen balance than do like rations carrying whole casein."

In view of the above question, additional evidence for the requirements of amino acids by humans has been considered. For infants, the values summarized in table 2 represent

Table 2

Calculated Amino Acid Intakes (mg/kg) of Infants on Natural Diets Compared With Requirements Directly Determined

Reference	Daily Requirement	Calculated Daily Intake				
	Table 1	*27*	*116*	*5*	*53*	*25*
Histidine	32	21	24	63	70	44
Isoleucine	90		75	90	170	110
Leucine	150		135	425	265	205
Lysine	105	83	83	170	144	131
Methionine	85	30	32	86	67	41
Phenylalanine	90		61	169	180	85
Threonine	60		51	87	112	98
Tryptophan	22	31	16	30	38	40
Valine	93		80	161	179	110

the amino acid composition of the protein determined to be required for normal growth. It will be noted that the minimal amino acid intakes reported under references *5, 53,* and *25* on natural diets are considerably in excess of the minimal requirements as directly determined. In exception to this are the data of Holt and Snyderman (116) on milk protein, which indicate a normal performance on intakes slightly below the requirement figure. This may be attributed to the nature of the diet. The exception does not hold in the case of methionine.

For adults, a similar comparison is made in table 3. The minimal requirements indicated in table 1 are compared with minimal amino acid intakes from natural diets and from intakes of various proteins needed to establish nitrogen equilibrium. Again it appears that the minimal intakes of indispensable amino acids furnished by such diets are far in excess of the minimal requirements as directly determined.

The last two columns in table 3 represent a more precise attempt to relate the essential amino acid content of proteins to their nutritive performance. The method of Harte and Travers (107) was used together with the more recent analytical values for amino acids as summarized by Orr and Watt (164) to calculate minimal amino acid intakes. These calculated minimal quantities are compared with similar values proposed by Mitchell (156). It is apparent that, with the possible exception of the sulfur-containing amino acids, all of the predicted minimum requirements as derived from studies of the amount of protein needed to maintain nitrogen equilibrium are above the minimum requirements listed in table 1.

As a consequence of this latter comparison, several questions are obvious. First, how realistic are the minimum values summarized in table 1? Second, in view of the improved methods now available for amino acid analysis (38), the availability of better estimates of the amino acid content of foods (164), and the close correlation of the essential amino acid contents of food proteins and their Biological Value (156),

Table 3

Calculated Amino Acid Intakes of Adults (g/day) from Experimental Diets as Compared with Minimal Requirements

Daily Requirement			Calculated Minimal Intakes								Minimal Intake	Est. Req. (156)
			From mixed diets				From nitrogen balance experiments [1]					
Reference	Table 1		(*113*)	(*176*)	(*207*)	(*207*)	Whole Egg	Milk	Soy Flour	White Flour		
	Male	Female										
Histidine	—	—	—	1.77	1.51	1.56	0.68	0.66	**0.60**	0.85	0.60	0.55
Isoleucine	0.70	0.45	1.39	3.76	3.51	3.31	1.92	1.61	**1.35**	1.85	1.35	1.12
Leucine	1.10	0.62	1.12	5.27	5.36	5.29	2.53	2.49	**1.96**	3.12	1.96	1.86
Lysine	0.80	0.50	1.23	5.69	4.07	3.57	1.77	1.96	1.60	**0.89**	0.89	0.99
Methionine+ Cystine	1.01	0.55	—	2.15	—	—	1.53	0.84	**0.80**	1.35	0.80	1.05
Phenylalanine+ Tyrosine	1.40	1.12	—	7.93	—	—	2.88	2.51	**2.06**	3.50	2.06	2.42
Threonine	0.50	0.305	1.07	2.37	2.86	2.56	1.39	1.15	**1.00**	1.23	1.00	0.56
Tryptophan	0.25	0.157	0.24	0.65	0.66	0.69	0.47	0.36	**0.35**	0.49	0.35	0.32
Valine	0.80	0.65	1.07	4.19	3.79	3.76	2.06	1.74	**1.33**	1.85	1.33	1.18

[1] Calculated according to (107) from protein requirements for nitrogen equilibrium (19) utilizing the amino acid composition data of (164).

is not the type of procedure summarized in table 3, or one of a similar type, more useful for practical nutrition evaluations? It is evident that the amino acid requirements are in the main dependent on the composition and pattern of the amino acids in the tissues being formed during growth or maintained in adult life (6, 156). The striking similarity in the amino acid composition of animals and its relationship to the pattern of amino acid requirements as determined by nutritional studies has been noted (249).

Inasmuch as the utilization of amino acids for the synthesis and maintenance of tissue proteins is an "all or none" process, it would appear that the use of a properly determined pattern of amino acid requirements in conjunction with nutritional evaluations of protein needs and modern methods of amino acid assay should provide a sound basis for the formulation of practical diets that will promote the desired nutriture of humans. The question remains as to the best reference pattern to employ.

Amino Acid Proportionality Patterns

Because of the remarkable similarity in the relative proportions of amino acids required among animals in general (6, 80, 156, 249), and among humans in particular, both for growth and adult maintenance (table 4), an ideal reference pattern could be one which represents the limiting value for each indispensable amino acid. A pattern is presented in column 1 of table 5 based on the average male and female patterns of adults from table 4.

The utility of the proportionality pattern may be observed from the data presented in columns 2, 3, and 4 of table 5. The figures in column 2 represent milligrams of amino acids in a derived protein providing 250 mg of tryptophan (the minimum daily requirement of the adult male) and the other amino acids in accord with their respective proportionality factors. The figures in columns 3 and 4 of table 5 illustrate the amounts in milligrams of amino acids supplied by the amount of whole egg and cow's milk protein, respectively, that will supply 250 mg of tryptophan (164).

The comparative figures for whole egg protein in table 5 indicate that all of the indispensable amino acids are in excess of the indicated requirements except the total sulfur-containing and the total aromatic

Table 4

Proportionality Patterns of Amino Acid Requirements of Humans (Tryptophan = 1.0)

	Infants (from Table 1)	Adults (from Table 1)		Adults (from Table 3)	
		Female	Male	Minimal Intake	Estimated from (*156*)
Histidine	1.5			1.7	1.7
Isoleucine	4.1	2.9	2.8	3.9	3.5
Leucine	6.8	3.8	4.4	5.6	5.8
Lysine	4.8	3.2	3.2	2.5	3.1
Methionine	3.9 3.0	2.2	4.4 0.8		
Cystine		1.3	3.2		
Total Sulfur A.A.		3.5	4.4 4.0	2.3	3.3
Phenylalanine	4.1	1.4	4.4 1.2		
Tyrosine		5.7	4.4		
Total Aromatic A.A.		7.1	4.4 5.6	5.9	7.6
Threonine	2.7	1.9	2.0	2.9	1.8
Tryptophan	1.0	1.0	1.0	1.0	1.0
Valine	4.2	4.1	3.2	3.8	3.7

amino acids. The discrepancy with respect to the latter appears to be insignificant. However, for the total sulfur-containing amino acids the deficit is 12.5 per cent, and, although 15.2 gm of whole egg protein will supply 250 mg of tryptophan, it will require 17.4 gm (15.2÷0.875) to furnish the indicated need for the sulfur-containing amino acids.

A similar comparison for cow's milk protein indicates that the sulfur-containing amino acid deficit is 37.7 per cent, and 28.4 gm (17.7÷0.623) of cow's milk protein is required to supply all of the indicated requirements.

On the basis of data in table 5, therefore, whole egg protein in the amount of 17.4 gm (or 2.78 gm of total nitrogen) and cow's milk protein in the amount of 28.4 gm (or 4.45 gm of total nitrogen) should be adequate to supply the indispensable amino acids required for male adult maintenance providing the protein was completely digestible and the amino acids wholly available. For a 70 kg adult, this represents 0.25 gm of egg protein per kg (0.040 gm of total egg nitrogen per kg) and 0.41 gm of cow's milk protein per kg (0.064 gm of total cow's milk nitrogen per kg).

Bricker, *et al.* (42), in their studies of the protein requirements of adult humans, found that 9 young female adults with an average weight of 60 kg and an average basal metabolism of 1300 calories per day required 2.76 mg of nitrogen per basal calorie and 22.4 gm of protein per day for nitrogen equilibrium when milk was the source of protein. Thus, a total of 3.59 gm per day of nitrogen and 22.4 gm per day of milk protein are fairly comparable to the calculated values per kg ($60\times0.41=24.6$ gm of protein or $60\times0.064=3.84$ gm of nitrogen).

Comparable data are not available for

Table 5

Amino Acid Proportionality Pattern for the Adult and Its Comparison with Egg and Milk Proteins

	1	2	3	4
	Proportionality Pattern of Amino Acids for Adults	Adult Male Amino Acid Requirements	Amino Acids Supplied by [1]	
	(Tryptophan = 1.0)	(Tryptophan = 250 mg)		
			Whole [2] Egg	Cow's [2] Milk
			mg/day	
Isoleucine	2.8	700	1008	1131
Leucine	4.2	1050	1337	1740
Lysine	3.2	800	972	1379
Total Sulfur A.A. (Methionine-minimum = 1.3)	3.8	950 (Methionine-325)	**831**	**592**
Total Aromatic A.A. (Phenylalanine minimum = 1.3)	6.2	1550 (Phenylalanine-325)	**1531**	1763
Threonine	2.0	500	756	817
Tryptophan	1.0	250	250	250
Valine	3.6	900	1128	1218

[1] From reference (164)

[2] Whole egg protein contains 103 mg of tryptophan per gram of total nitrogen. It will require 2.43 gm of total whole egg nitrogen or 15.2 gm (2.43 × 6.25) of whole egg protein to furnish 250 mg of tryptophan. Similar values for cow's milk protein are 2.78 gm of total nitrogen and 17.7 gm (2.78 × 6.38) of protein.

whole egg protein, but a rough comparison can be made by utilizing the determined Biological Values of the proteins of milk and egg (156). These are given as 74 and 94, respectively, which indicate that milk protein B V is 79 per cent that of egg protein. Utilizing this value, the amount of egg protein required for nitrogen equilibrium would be $0.79 \times 28.4 = 22.4$ gm per day (or $0.79 \times 4.45 = 3.52$ gm of total nitrogen). It will be recalled that the figures calculated from the amino acid reference pattern for egg were 17.4 and 2.78 gm respectively.

The Protein Requirement

In assessing the protein requirements of man, or of any animal, the quantitative demands as well as the qualitative needs must be considered. The reference proportionality pattern of amino acids as developed in the previous section appears to offer a sound basis for meeting the qualitative requirements. If a satisfactory basis can be found for meeting the quantitative needs of humans, not only for maintenance but also for growth and other physiological demands, it should be possible by combining these two parameters to arrive at a tentative basis for defining adequate protein nutriture in humans and for practical measurement of the protein adequacy of the food supply of various population groups.

The protein required by the body varies at different periods of life. At all times nitrogen is required for maintenance. Additional nitrogen is required for growth and maturation.

The quantitative protein requirements of humans have been studied extensively. Much of the pertinent literature has been summarized in the first part of this report and in two recent publications (110, 241). It is evident that the major problem revolves around "the criteria to be used for judging whether a given intake is adequate or not" (241). The term "adequate" encompasses not only the physiological requirement which includes growth and maintenance of protein equilibrium, but also a "hygienic requirement" (Terroine (241))—"the amount needed under the stresses of everyday life."

The requirements for protein for the different purposes noted above can be determined by direct observation and can be calculated on the basis of certain premises. Unfortunately, the direct observations are relatively few and confined mostly to the period of infancy and to adults. More observations are much to be desired. For the present it appears that one of the most objective approaches to estimating minimum nitrogen (or protein) requirements is the procedure emphasized by Hegsted (110) who made calculations of the requirement of an ideal protein at various ages based on certain assumptions: For maintenance, the fact that animals catabolize approximately 2 mg of nitrogen or 12.5 mg of protein per calorie of basal metabolism utilized. For growth, 18 per cent of the weight gain is taken as the protein increment. For fecal losses, an additional allowance of 10 per cent is added.

Minimum protein requirements based on the foregoing assumptions and derived for the classification of individuals according to the Recommended Dietary Allowances (82) are presented in table 6. The formula $P = 0.88 W^{0.734}$ when W = weight in kilograms and P = protein requirement per day in grams, taken from Smuts (211), was used to calculate the human basal requirements for protein (column 3). The requirements for growth, calculated on the basis that 18 per cent of the gain is protein, are presented in column 4. These values plus an addition of 10 per cent to cover fecal losses are totaled in column 6 to arrive at a minimum requirement. The values so listed represent the minimum requirements based on a provisional protein having a Biological Value of 100, or an amino acid pattern comparable to that proposed in table 5. Thus, an adult man would require 0.32 gm of the provisional protein per kg and an infant under one month of age would require 1.76 gm per kg.

The adjustment of the calculated minimal requirement for proteins with Biological Values less than 100 is illustrated in columns 7 and 8 for cow's milk protein (B V = 79) and for a diet in which the protein has a

B V of 60. It is recognized that there are limitations in attempting to cover amino acid deficiencies of a poor quality protein by large increases in the amount of protein given. The digestibility of the protein and the availability of the amino acids are primary considerations which are not evident from amino acid analysis. From a practical basis (50, 110, 241) it would appear that any protein or protein mixture having a protein utilization of 60 per cent or better would satisfy nitrogen and amino acid requirements when fed in sufficient amounts. In general, feeding a protein of the above efficiency at the proper level to an adult would be practical and probably under most circumstances would not involve any amino acid imbalance. However, when dealing with protein efficiencies of 60 per cent or less, proper supplementation either by selected protein foods or by judicious addition of limiting indispensable amino acids

Table 6

Calculated Minimal Protein Requirements of Humans as Grams per Kilogram of Body Weight [1]

	1	2	3	4	5	6	7	8
	Age (yrs)	Wt (kg)	Basal Req.	Growth Req.	10% of (3 + 4) as fecal loss	Total Min Req.[2]	Cow's Milk B V=79 [3] (Factor = 1.266)	Limiting Diet B V=60 [3] (Factor = 1.667)
Men	25	70	0.28	0.01	0.03	0.32	0.41	0.53
	45	70	0.28		0.03	0.31	0.39	0.52
	65	70	0.28		0.03	0.31	0.39	0.52
Women	25	58	0.30	0.01	0.03	0.34	0.43	0.57
	45	58	0.30		0.03	0.33	0.42	0.55
	65	58	0.30		0.03	0.33	0.42	0.55
	Pregnant (2nd half)		0.30	0.10	0.04	0.44	0.56	0.73
	Lactating (850 ml. daily)		0.30	0.21	0.05	0.56	0.71	0.93
Infants	Birth to 3/12	4.55	0.59	1.01	0.16	1.76	2.23	2.93
	3/12 to 6/12	6.65	0.53	0.57	0.11	1.21	1.53	2.02
	6/12 to 9/12	8.35	0.50	0.36	0.09	0.95	1.20	1.58
	9/12 to 12/12	9.60	0.48	0.21	0.07	0.77	0.97	1.28
Children	1-3	12	0.45	0.04	0.05	0.54	0.68	0.90
	4-6	18	0.41	0.08	0.05	0.54	0.68	0.90
	7-9	27	0.37	0.08	0.05	0.50	0.63	0.83
	10-12	36	0.34	0.06	0.04	0.44	0.56	0.73
Boys	13-15	49	0.31	0.07	0.04	0.42	0.53	0.70
	16-19	63	0.29	0.04	0.03	0.36	0.46	0.60
Girls	13-15	49	0.31	0.07	0.04	0.42	0.53	0.70
	16-19	54	0.30	0.02	0.03	0.35	0.44	0.58

[1] See text for details. The groupings used in formulating the table, except for infants, were those used by the Food and Nutrition Board, National Research Council, Recommended Daily Dietary Allowances, Revised 1958 (82). For infants, the age and weight values were taken from table 7 of the 5th edition of the Mitchell-Nelson Textbook of Pediatrics (162). The 50th percentiles for boys were used throughout. The weight value for birth to 3 months (4.55 kg) is an average of the birth weight (3.4 kg) and the weight at 3 months (5.7 kg), etc.

[2] The values in column 6 represent calculated minimal requirements of a provisional protein with a Biological Value of 100, or an amino acid proportionality pattern comparable to that in table 5.

[3] *These are calculated minimum values and not recommended allowances.* Any recommended allowance should consider individual variability and allow for the specific needs of a particular population under varying conditions.

would appear to be warranted in order to avoid amino acid imbalance.

Where attempts have been made to apply a reference amino acid pattern in estimating quantitative protein needs, it is evident that the procedure has practical utility (50, 241). It is also apparent that such a procedure can serve only as a rough guide at the present stage of our knowledge of the nitrogen and amino acid needs of the human population. Not only is there a need for more exact definition of the nitrogen requirements throughout the life span; there is a more urgent need for evaluating the so-called "hygienic demands" (241). Consonant with the latter is the requisite for increased precision of amino acid assay and evaluation of protein efficiency.

In an attempt to establish a yardstick for human nutrient requirements, it should be obvious that the values proposed are not only minimums, but, more important, they are averages. Furthermore, the averages are subject to additional restriction inasmuch as they are derived from selected experimental subjects. Consequently, such values are only useful as a guide for practical nutrition and are not, or should not be, considered as standards. Practical nutrition requires art as well as science, since individuals as well as population groups are subject to physiologically changing requirements (climate, etc.) and pathological stresses (infections, parasites, etc.) which demand a flexibility that is not inherent in a set of minimum values. In the meantime, the principles outlined herein may serve as a tentative procedure for attacking the practical problems of the protein nutriture of humans.

Effects of Deficiency of Proteins and Amino Acids

Much of our knowledge concerning the roles of protein and amino acids in nutrition has been obtained from studies of deficient states associated with deprivation of the particular material under study. Hence, it would appear desirable to describe certain well-established effects of protein and amino acid deficiencies. The data have been obtained from two sources: experimental animals and man. Much more information is available concerning the former group, particularly the rat, in which deficiency may be produced at will by dietary restriction. With the exception of the studies on requirements of children and adults which are discussed in the preceding section, much of our information has been obtained from observations on naturally occurring syndromes in which, since evidence of protein depletion is usually present, it may be conjectured that protein and/or amino acid deficiencies play some role in their pathogenesis. Because of the lack of similarity between artificially produced (usually single-nutrient deficiency states) and naturally occurring, multiple-nutrient deficiency syndromes, it seems logical to discuss them separately. Hence, this section will be divided into two parts: (1) *Effects of Protein and Amino Acid Deficiencies in Experimental Animals* and (2), *Syndromes Associated with Protein Depletion in the Human.*

Effects of Protein Deficiency in Experimental Animals

As has been indicated elsewhere, observations made during the past 150 years have clearly established that in experimental animals protein deficiency must be studied from two standpoints: deprivation with respect to *quantity* and with respect to *quality*. In regard to *quantity,* protein deficiency as a whole will be discussed; to consider *quality,* the effects of deprivation of single amino acids need to be delineated.

When the protein intake of an experimental animal (rat, mouse, guinea pig, dog, pig, etc.) is restricted, certain well-marked biochemical and anatomical changes may be observed. All such abnormalities naturally reflect alterations in the protein composition of the cells as well as in their extracellular products. Changes in the latter, of course, merely indicate biochemical lesions in the former and thus serve as an index with which to study cellular function.

When an animal is placed on a protein-deficient diet, a number of striking changes may be noted. If the experimental subject is in its growing period, growth may be retarded or cease completely, or even weight loss may ensue. If the subject has attained its full growth, only weight loss may be encountered. If the animal has reached sexual maturity, reproductive activity, in both the male and the female, may be retarded or completely abolished.

Before going on to detail some of the changes which are encountered in protein-deficient animals, certain points of a general nature should be mentioned. First, it is important to indicate the difficulty of differentiating the precise effects of protein deficiency *per se* from those of caloric restriction. Few pointed studies have been carried out with this in mind; such are highly desirable, particularly for the light which they might throw on human protein depletion syndromes. One must always be aware of the nonspecific effects of caloric restriction. Anyone who works in this field will find *The Effects of Inanition and Malnutrition on Growth and Structure* by C. M. Jackson

(121) and *The Biology of Human Starvation* by Ancel Keys and his collaborators (126) of inestimable value.

Another critical point, which has been considered far too infrequently in the past and continues to be neglected even today, is the matter of controls. Three methods have been utilized in studies of dietary deficiencies: (a) *ad libitum* feeding, (b) paired feeding, and (c) paired weight gain feeding. Each of these methods has its value, depending on the type of information being sought. For the comparison of structural alterations in the tissues, when it is important to detect the nonspecific effects of inanition, the paired weight gain technique is to be preferred.

Finally, it is important to realize that the degree of deficiency that is produced may have an important bearing on the appearance and course of any pathologic changes. Acute, complete protein deficiency leads to relatively little change save growth failure and atrophy of the tissues. On the other hand, less severe forms of deficiency, which may permit some biochemical activities as well as growth and reproduction of certain tissues, may provide much more information with respect to the metabolism and structure of cells and their extracellular products.

In considering alterations which may be observed in protein- or amino acid-deficient animals, it is necessary to bear in mind the difficulty, if not impossibility, of separating the effects of protein deprivation from caloric restriction. The degree of deficiency will, of course, modify the final picture.

Skeleton

As has already been indicated, growth of the protein-depleted organism may be greatly slowed; hence, profound alterations may be encountered in the skeletal tissues (78, 84). Depending on the severity of the deficient state, retardation of cell proliferation in the epiphyseal or costal cartilages will be found. These areas are a critical index of the nutritive status of the organism. The changes which take place in cartilage are nonspecific and differ in no wise from those which occur as the result of caloric restriction or deficiencies of any one of the 40-odd essential nutrients (79). Similarly, osteoblastic activity is greatly retarded, so that relatively little formation of periosteal and endosteal bone may be observed. This again is a nonspecific reaction. In addition to the cessation of bone growth, the skeleton becomes rarefied. The latter also occurs in the adult, where endochondral bone growth has ceased (72). Evidences of disturbance in activity of the cells of the peridontal tissues and of the teeth themselves may be observed in protein-depleted animals (119).

Connective tissue cells

A cell which is closely related to those of cartilage and bone is the fibroblast; this is responsible for the elaboration of an important fibrous protein, collagen. One way of studying the activity of fibroblasts is to observe the elaboration of new collagen during the healing stages of artificially produced wounds. In protein-depleted animals, wound healing is markedly affected because of failure of collagen to be elaborated (236).

Reproductive tract

In view of the effects on reproductive activity, it is not surprising to find evidences of atrophy of tubules of the testes and impaired maturation of the follicles in the ovaries (163). As might be expected, there are evidences of lack of stimulation by the hormones produced by these structures on the various accessory sex organs.

Endocrines

In association with changes in the gonads, alterations have been noted in other endocrine glands. The hypophysis and thyroid (126) show atrophy. On the other hand, the adrenal may exhibit increase in width of its cortex (51). Such a change is associated with an overall diminution in the amount of lymphoid tissue. Hence, the thymus, lymph

nodes, and spleen are all reduced in size (79).

Skin

The epidermis may become atrophic; there may be some thinning and even loss of hair, although the latter may not be related to protein deficiency in general but to a lack of one or more essential amino acids (140). This aspect needs further study.

Eye

Protein deprivation effects the integrity of the cornea and lens. In the former, tissue vascularization, having no apparent relationship to any specific amino acid, may be prominent (229). The change is similar to that following deficiencies of single inorganic elements or vitamins. Cataract has been observed in protein depletion states and as the result of a single deficiency of most of the essential amino acids (97). Again this alteration does not differ from that seen in deprivation of a vitamin, such as riboflavin.

Pancreas

The glandular epithelial cells of the pancreas in protein-depleted animals exhibit a decrease in the amount of cytoplasm and a reduction in the number of granules which represent their secretory products (94). Hence, the pancreatic enzyme content of the intestine is lowered.

Kidney

The renal cells in protein-deficient rats become atrophic, with no other particularly striking change (121, 126).

Nervous tissues

No alterations are seen in the nervous tissues of protein-deficient animals save failure in development during growth phase (121, 126).

Muscle

Atrophy of cardiac, skeletal, and smooth muscle fibers is seen in protein-depleted animals (126). No other specific effects on muscle have been recorded.

Bone marrow and blood cells

Among the most studied effects of protein depletion is the decrease in hemoglobin production and hence in the number of red blood cells (142, 206). The erythropoietic elements in the bone marrow are usually hyperplastic. It is clear that certain amino acids may effect regeneration of hemoglobin better than others, yet the "all or none law" operates here just as it does in other tissues. Pointed studies of the white blood cells in protein-depleted animals have not been carried out.

Liver

The most extensively studied tissue in protein-depleted animals has been the liver. This is, of course, because of the numerous functions performed by this tissue, particularly with respect to synthesis of plasma proteins. When the liver from protein-deficient animals is studied microscopically the cells may, in the first few days, contain more fat than normal (128). The ribose nucleic acid content falls subsequently, hence the amount of basophilic material in the cytoplasm is decreased. As might be expected, changes in the concentrations of any number of enzymes may be demonstrated (127, 238). Finally, decreases in the concentrations of fibrinogen, albumin, and some of the globulin fractions of the serum occur and may become marked (151).

Immune responses

A complicated, though important, consequence of protein deficiency is its relation to the immune reaction. Decreases in antibody titer and alterations in the natural course of experimental infections have been demonstrated in animals placed on protein-deficient diets (44, 60). The problem ap-

pears even more complex when the animate agent is a virus, i.e., intracellular (220).

Effects of Amino Acid Deficiency in Experimental Animals

Soon after all of the essential amino acids had been delineated, in the late 1930's, they became available in quantities sufficient for studies of single deficiencies in experimental animals. At that time it seemed not too naive to hope that many interesting biochemical and anatomical defects might come to be ascribed to a deficiency of each of the essential amino acids. It soon became clear, however, that such a view was contrary to the "all or none principle" of amino acids in protein synthesis. Hence, although single deficiencies of each of the essential amino acids have now been studied, results other than the nonspecific effects of protein deficiency, described above, have been meager. However, investigations of this type should not be abandoned. Now that more has been learned of the intermediary metabolism of the essential amino acids, the time would appear propitious to attempt to delineate certain biochemical alterations which might be expected to result from their absence. Moreover, since deficiency of one doubtless alters the metabolism of others, partition of the essential amino acids in blood and urine might be worthy of study. Finally, examination of the tissues must not be neglected. Most of the reports thus far have dealt with complete deficiency of a single amino acid. Perhaps, incomplete deprivation might result in less debilitation from protein deficiency; and specific anatomic lesions might appear.

In the studies of the essential amino acids thus far reported, alterations have been found in all of the anatomical areas which have been mentioned in the preceding part of this section. It does not seem necessary to detail the reports of the studies of each of the essential amino acids which have now been carried out. Mention will be made of only those amino acids, a deficiency of which leads to a specific effect. These are tryptophan, lysine, isoleucine, threonine, methionine, and valine.

Tryptophan

In addition to a host of nonspecific effects, tryptophan deprivation leads to the following: niacin deficiency (52), alopecia (rat) (7), loss of pigment in incisor teeth (rat) (7), necrosis of muscle (skeletal, swine; cardiac and smooth, rat) (1, 45), and fatty liver (1). Tryptophan is the precursor of niacin in those species which have been studied, including man (52, 63, 90). Hence, this amino acid has come to occupy the same position with respect to this vitamin as methionine holds in relation to choline. The effects of tryptophan deficiency have yet to be fully investigated in the presence of adequate amounts of nicotinic acid. Therefore, the alopecia (7), loss of pigment of the teeth (7), and necrosis of muscle (1, 45) may be due to nicotinic acid deficiency rather than to deprivation to tryptophan *per se*. As for the fatty infiltration of the liver (1), tryptophan is one of several amino acids leading to this change, which primarily affects the periportal parts of the hepatic lobule.

Lysine

Deprivation of this amino acid leads to two specific changes in the rat: achromotrichia (237) and fatty liver (208). The pathogenesis of the alteration in hair color is not known. The fatty liver is of the periportal type.

Isoleucine

Studies of isoleucine-deficient rats have revealed necrosis of skeletal muscle (203). This observation needs to be confirmed.

Threonine

Large amounts of periportally distributed lipid are found in the livers of threonine-deficient rats (209).

Methionine

This amino acid is a precursor of cystine, hence lack of it will give rise to both deficient states unless cystine is added to the diet. When methionine deficiency is initiated in the presence of adequate dietary cystine, fatty liver results unless choline is added to the diet (79). In the presence of choline, methionine deficiency results only in the poor formation of hemoglobin and plasma proteins (88). With suboptimal amounts of methionine in the diet and no added cystine, acute necrosis of the liver occurs (88). This is made worse by lack of tocopherol (96) and a third material, Factor 3, much of the activity of which is associated with selenium (201). Because of the high content of cystine in hair, it is not surprising to find derangements of hair growth in animals deficient in this amino acid (211).

Valine

Some years ago convulsions were reported in rats whose diet was deficient in valine (178). These observations have been confirmed by others (73).

* * *

It will be noted that a deficiency of at least three of the amino acids (tryptophan, lysine, and threonine) leads to fatty infiltration of the liver. The degree of such alteration in lipid content depends on the degree of deficiency, i.e., partial restriction produces more extensive changes than does total deprivation.

This section has dealt with the effects of protein and/or amino acid undernutrition. The results of excesses or imbalances of amino acids are discussed in the section to follow. Studies of the effects of excess protein on the function and structure of organs and tissues in experimental aminals have been and will continue to be pertinent as far as the human is concerned.

Syndromes Associated with Protein Depletion in the Human

Protein depletion may be brought about in the human by a number of mechanisms. These include: defective intake with respect to *quantity* or *quality*, poor absorption, inordinate loss, excessive destruction, and faulty synthesis. This presentation will be concerned with those disease states in which defective intake probably plays the major role, although other factors such as poor absorption or excessive loss in the presence of diarrhea, faulty synthesis as a result of liver disease, etc., may add to the primary difficulty.

There are four important human syndromes in which evidence of protein depletion are prominent and in which protein deficiency appears to play a role. These are hunger edema, pellagra, kwashiorkor, and nutritional liver disease. Only one of these, nutritional liver disease, is of any consequence today in this country. The others are of importance throughout the rest of the world, although at the present writing the precise extent of their prevalence is not known.

Hunger edema

During and following World War I, in association with the extreme malnutrition which was observed in central Europe, edema came to be recognized as a familiar accompaniment of semistarvation (126, 146). Decreases in plasma protein concentrations were soon demonstrated; particularly prominent were reductions in the albumin fraction of the serum. Soon, too, studies in animals demonstrated that dietary protein restriction would lead to hypoproteinemia. During the 1920's and 30's it was generally taught and believed that protein depletion and hunger or starvation edema were virtually synonymous. The historical aspects of this subject are adequately summarized elsewhere (146).

With the advent of World War II and the reappearance of mass semistarvation in civilian populations and among the inmates of concentration and prisoner of war camps, the problem of the relation of dietary protein deficiency to edema again came into prominence; consequently, it received pointed study in its naturally occurring form and in human volunteers in the experi-

mental laboratory (126). Such investigations utilizing modern methods clearly indicated that the problem in man was not nearly as simple as it had been thought to be during the preceding quarter of a century. A number of important factors, some of which appeared to be more cogent than dietary protein deficiency, were singled out and studied. To summarize, it is now clear that, in a given case of edema associated with caloric and protein malnutrition, one or more of the following factors may operate: the general increase in body fluid which in part replaces lost fat and cells; salt intake; posture; tissue tensions; vascular permeability and intravascular pressures; renal function; hormonal effects; and intake of non-protein foodstuffs. Varying degrees of protein depletion are evidenced by a lowering of the concentration of plasma proteins and play a role as well in the deranged metabolism of water. The above summary of the many factors which may be implicated in a given case of hunger edema serves to emphasize the complex nature of this human dietary deficiency state. Protein deficiency is only one of many factors giving rise to the excessive retention of fluid which is the most prominent manifestation of the hunger edema syndrome.

Pellagra

The characteristic clinical course of pellagra and the story of the development of our knowledge of what seemed to be its rational therapy are well known. After the demonstration of the effectiveness of nicotinic acid in the treatment of experimental black-tongue in dogs (69) and of pellagra in man (219), emphasis shifted to the role of the amino acid, tryptophan, in the pathogenesis of the disease. Now it has been demonstrated in a variety of species (52), including man, that tryptophan is a precursor of niacin.

A relation between maize and pellagra has been recognized for many years. It is now known, not only that corn is deficient in tryptophan, but further that some of its niacin content may not be readily available. Since the discovery of niacin and of the niacin-tryptophan relationship there has been a tendency to redefine pellagra as a deficiency of niacin-tryptophan (89). There is no doubt that experimental deficient diets will reproduce most of the symptoms of the disease and that the administration of these factors will prevent or cure it. Nevertheless, the naturally occurring syndrome of pellagra may be complicated by other deficiencies.

Although means are at hand to treat pellagra quite effectively, it is unfortunate that so little is known of the underlying biochemical derangements which lead to the anatomical features of the disease that is being treated.

Kwashiorkor

Since the initial description of the kwashiorkor syndrome twenty-five years ago (248), this nutritional disease of infants and young children has been shown to have worldwide prevalence, particularly in tropical and subtropical areas (122).

The syndrome of kwashiorkor generally consists (29, 99, 123) of the following: retardation of growth; weight loss; edema; muscular wasting; apathy and other psychic changes; dermatitis; changes in the hair; enlargement of the liver as a result of fatty infiltration; and protein disequilibrium as evidenced by alterations in protein concentrations of the plasma and in enzyme concentrations of plasma and gastrointestinal secretions. Two other features are important: The syndrome is seen most commonly after weaning and is usually precipitated by some stress, such as infectious disease or an episode of diarrhea.

Growth failure, weight loss, and edema can be looked upon as the basic manifestations of the syndrome. The other disturbances enumerated above are not necessarily present in every case, for it is apparent that certain factors, particularly the intake of protein with respect to quantity and quality, as well as calories modify the syndrome. One end of the spectrum of disease (29) is characterized by fatty liver, edema, and

hypoproteinemia, without striking skin or hair changes; this is seen *par excellence* as "sugar baby disease" in Jamaican infants, whose diets contain more than adequate calories but are deficient in protein (123). At the opposite end of the spectrum is found marasmus, that is, starvation; here growth failure and wasting of all tissues are the prominent manifestations resulting from a deficiency of all nutrients. The type of disease which is most prevalent today is marasmic kwashiorkor, which exhibits all gradations between "sugar baby disease" and maramus.

Marasmic kwashiorkor is dependent on a deficiency of poor quality protein and of calories. Recent studies in West Africa (115), South Africa,* and Central America (29) have brought out the dual role of protein and calories in the pathogenesis of marasmic kwasiorkor. The importance of protein is further evidenced by the effectiveness of crystalline amino acids in curing the disease (99).

Nutritional liver disease

During the past two decades, increasing evidence has been gathered on the role of nutritional factors in the pathogenesis of liver disease in the human. The early evidence was derived from studies in experimental animals, in which fatty change with or without cirrhosis was produced by choline-deficient diets, or acute hepatic necrosis was related to a lack of cystine, tocopherol, or Factor 3 (selenium). It is difficult, as might be expected, to transfer carefully controlled observations in the laboratory to the complex biochemical and structural alterations which contribute to the disease picture in man. The main benefit of the experimental studies has been to focus attention on nutritional liver disease in man; as a result, intensive biochemical and anatomical (biopsy) studies have been carried out during life, and careful observations of the structure of the liver in health and disease have been reported. There can be little doubt that protein deficiency in man may lead to liver dysfunction, although the precise pathogenesis of the changes is not always clear.

Nutritional liver disease in man is often seen in the alcoholic whose diet is poor; less frequently it occurs also in individuals who are losing or destroying protein at an abnormal rate or are failing to synthesize adequate protein for a variety of reasons. The typical alcoholic may maintain an adequate caloric intake, though his ingestion of essential nutrients, particularly protein, may be exceedingly low. Three syndromes which merge into one another may be encountered: simple fatty liver disease, fatty liver disease with hepatic failure, and nodular or Laennec's cirrhosis (170).

Simple fatty liver disease is frequently discovered incidentally in a known alcoholic or in an individual who upon questioning proves to be one. Besides an enlarged liver, which on biopsy is shown to contain much fat, relatively little else may be found. If the individual is returned to a normal diet, the fat disappears from the liver, not to return unless the dietary again becomes inadequate.

If a fatty liver such as the above persists for any length of time, one may expect to find evidences of hepatic functional changes, such as increased bromsulphalein retention, rise in serum bilirubin, hypoproteinemia, etc. At this stage the individual is ready to show the dramatic picture of acute liver failure, which frequently appears to be aggravated by an infection. When severe hepatic insufficiency has developed, the course is frequently difficult to change and death ensues. At autopsy, an excessively fatty liver is found, which may or may not show fresh necroses and usually displays relatively little excess connective tissue.

The third type develops initially as a fatty liver in which connective tissue slowly proliferates. As a result there is interference with the circulation and the liver cells are further damaged. Moreover, since blood flow is obstructed, the increased pressure in the portal system leads to derangements elsewhere, particularly the formation of collaterals and ascites. Liver failure may be the cause of death, or exitus results from

* J. D. L. Hansen. Personal communication.

rupture of an esophageal varix, infection, or general debility.

In patients whose liver disease falls into the first type (simple fatty liver disease), as has been mentioned, therapy may be effective. Such treatment consists of high protein diet with or without supplements of choline. This therapy is sometimes effective before failure has occurred in the second group, though frequently all therapy is of no avail. When cirrhosis has developed, one may only expect to arrest the development of the disease, since little can be done to alter the pattern of connective tissue in the liver. Hence, adequate diet is indicated. It is particularly in nutritional liver disease that the role of protein is so difficult to evaluate. Evidences of protein disturbance are seen in the concentrations of plasma proteins in the serum. Some of this is doubtless due to insufficient amino acids for protein manufacture, yet much doubtless results from defective formation of protein by a diseased liver.

There is little evidence to indicate that protein malnutrition is of any consequence in the normal population of the United States today, though so far too few studies have been carried out using the criteria which have been described elsewhere in this publication (page 35). More investigations are needed. The reports which have appeared dealing with dietary habits and the outcome of pregnancy (43, 61, 147, 245) are conflicting. So, too, the literature (59, 141, 172) on protein malnutrition in children needs amplification. Studies dealing with the need for supplementation of milk with lysine (8, 9) do not appear to stand critical evaluation, particularly since recent studies in Mexico (91) fail to show beneficial effects of adding lysine to milk.

This discussion of protein malnutrition in man so far has centered entirely on deficiency states resulting from a lack of nutrients from *exogenous* sources. It must be emphasized, however, that an increasing number of *endogenous* protein or amino acid deficiency states are coming to be recognized. These range from genetic disturbances in which the synthetic mechansim for a single protein enzyme is lacking to disturbances of the kidney, liver, and other organs in which some primary disease leads to loss or poor formation of proteins and/or amino acids. Among the many "inborn errors of metabolism" which are doubtless enzymatically controlled, certain are associated with protein and amino acid metabolism *per se*. A good example is phenylketonuria. Other genetically controlled disturbances result in amino aciduria of varying types. Still other examples of endogenous deficiency states are the hypoproteinemias associated with the nephrotic syndromes, chronic blood loss, long-standing infections, chronic diarrhea, or chronic hepatic disease, which last may have been initiated by protein depletion in the first place.

When the subject of protein and amino acid deficiency in man is looked upon from the standpoint of both exogenous and endogenous factors, it naturally becomes more complex. Yet it is just this complexity which must be unraveled if we are to understand naturally occurring protein and amino acid deficiencies in the human.

In regard to dietary surfeits data are not adequate to determine whether excessive protein intake is deleterious for a normal population or what the safe upper limit, if any, might be. Even in diseased states such as those associated with renal insufficiency, hypertension, etc., in which the clinician may advise dietary protein restriction, the rationales need stronger foundations.

Effects of Disproportionate Amounts of Amino Acids

Supplementation of the diets of animals with crystalline amino acids has been a common practice in laboratory experiments since the nutritional importance of these building blocks of proteins was recognized over fifty years ago by Willcock and Hopkins (247) and Osborne and Mendel (165). The original experiments of these two groups provided indisputable evidence that the nutritive value of many proteins could be improved by the judicious use of amino acid supplements. Their conclusions to this effect have been amply confirmed and greatly extended (74). However, the many cases in which amino acid supplements have been used with success should not be allowed to obscure the fact that there are conditions under which the addition of amino acids to a diet may cause an adverse rather than a beneficial effect (102). Many of the conditions under which such adverse effects have been observed would not be encountered in practical nutritional situations. Nevertheless, the fact that such effects can be demonstrated, and the realization that our understanding of the basis for them is limited, are adequate reasons for discussion of this subject and emphasize the need for further study of it.

Certain amino acids, in quantities that greatly exceed the requirement, are not well tolerated by experimental animals. Among the earliest reports of such effects are those of Lewis (138) and Sullivan, Hess, and Sebrell (223). Adverse effects have since been observed in animals fed (102, 103, 198) or injected (95, 198) with relatively large quantities of a variety of individual amino acids, and under certain conditions when the proportions of the amino acids in a diet have been altered by adding relatively small quantities of one or a few amino acids. The effects of injections of amino acids have recently been studied methodically (95) but the adverse effects which arise from additions of large quantities of individual amino acids to diets or from the creation of amino acid imbalances in diets have not been as methodically investigated. There is still some question regarding the way these effects should be classified (102, 197), that is, whether they should all be included as manifestations of imbalance or whether there are sufficient grounds for separating them in some way.

As more evidence accumulates the effects of administered amino acids appear to fall into distinct categories among which the following may be recognized: (a) *Amino acid toxicities.* These appear to result from a failure on the part of the animal to metabolize an excess of an amino acid sufficiently rapidly to prevent the accumulation of either the amino acid itself or some metabolic product of it which causes an adverse effect. Such effects can be demonstrated in animals consuming diets that contain adequate quantities of the known required nutrients, diets that would otherwise support normal growth. In some cases the adverse effect can be at least partially alleviated by administering a substance which facilitates the catabolism of the excessive quantity of the amino acid and which appears to play the role of a detoxifying agent. (b) *Amino acid antagonisms.* These appear to fall within the classical concept of metabolic antagonists, in which a structural analogue of an essential dietary component competes with a dietary essential for a site on an enzyme or an absorptive mechanism or becomes incorporated into a product of metabolism, producing a biologically inert instead of a biologically active substance. (c) *Vitamin relationships.* Ad-

verse effects from amino acid additions have been observed in animals consuming diets which contain an inadequate quantity of one of the vitamins known to be involved in amino acid metabolism. In these cases, provided the amino acid is not administered in quantities sufficient to produce a toxic effect, an increased intake of the limiting vitamin prevents the adverse effect. (d) *Amino acid imbalances.* These have been demonstrated primarily in experiments on animals consuming insufficient protein to support normal growth. A relatively small increase in the dietary level of one or more amino acids may so alter the amino acid proportions as to cause a retardation of growth or some other abnormality. Increasing the dietary levels of the amino acids which are most limiting for growth counteracts these effects.

Effects of Administering Excessive Amounts of Individual Amino Acids

Toxic effects

Adverse effects resulting from the administration of excessive quantities of certain of the vitamins, particularly vitamins A and D, and a number of the required trace elements have long been recognized and have been referred to as vitamin and mineral toxicities. The administration of large quantities of certain amino acids to experimental animals will cause severe growth retardations (102, 103, 198) and, depending upon the mode of administration and the nature of the basal diet, can cause death (95, 198). These effects have been studied in detail in only a few cases, so it is perhaps not justifiable to describe them all as toxic effects. However, enough information has been obtained in a few cases to suggest that some of these are sufficiently analogous to vitamin and trace mineral toxicities to be classed as amino acid toxicities.

Parenteral administration. The earliest evidence that the administration of relatively large quantities of individual amino acids to animals might produce toxic effects was obtained from experiments in which the amino acids were injected intravenously or intraperitoneally (138, 198). There have since been a number of reports of such effects arising from the injection of many individual amino acids (198). The most complete report is that of Gullino and associates (95), who demonstrated that each of the essential amino acids produced severe toxic reactions when administered intraperitoneally in sufficient quantity to animals in the fasting state. They determined the LD_{50} and $LD_{99.9}$ dosage for each of the ten essential amino acids and demonstrated that, within limits, the toxicity could be alleviated by administering L-arginine prior to, or simultaneously with, a toxic dose of the other amino acids. The toxicity in the absence of L-arginine was related to the accumulation of ammonia; nevertheless, since the amino acids differed appreciably in toxicity and in the symptoms they produced, the possibility that there might also be specific effects which depend upon the individual amino acid structures was considered.

The significance of these observations with regard to intravenous alimentation appears obvious and will undoubtedly be investigated in detail. Their significance with regard to the effect of oral administration of large amounts of individual amino acids is less evident. In a few experiments in which L-arginine was included in diets containing an excessive amount of some amino acids no beneficial effect was observed.* However, a more detailed investigation of the effect of arginine in feeding trials is desirable and might contribute to our understanding of the way in which adverse effects from feeding excessive amounts of individual amino acids are brought about.

Oral administration. The amount of an individual amino acid which must be ingested in order to produce an adverse effect is not a constant but varies greatly with the nutritional regimen employed. There are also great differences among the amino acids, some being tolerated in much larger quantities than others. In view of the relatively meager knowledge we have of the basis for these effects, one of the main difficulties in classifying the adverse effects resulting from the ingestion of excessive quantities of indi-

* A. E. Harper. Unpublished data.

vidual amino acids is to know where to draw he line between what can justifiably be alled a toxic effect and what should be mitted from this grouping. Should a slight growth depression which is attributable to an ncrease in the intake of a normal dietary onstituent be described as a toxic effect? Should a severe growth depression accompanied by lesions be so described? Should it depend upon the mechanism by which the effect is produced? And what if there is, as yet, no satisfactory evidence concerning the mechanism?

It is impossible to resolve this dilemma with our present knowledge. However, some of the adverse effects obviously fall into specific categories while others cannot be readily characterized. In an attempt to bring some order out of the chaos, adverse effects resulting from the ingestion of large quantities of individual amino acids, and which do not at present appear to fall within any of the other general groupings that have been recognized, have been classed as amino acid toxicities.

Two examples appear to fall definitely within this category. An intake of methionine just above double the requirement will retard the growth of rats, an effect which becomes more severe as the level of methionine in the diet is increased. The growth retardation can be demonstrated in animals fed on diets containing adequate quantities of all known nutrients; however, the effect may be more severe if the diet contains inadequate quantities of protein or pyridoxine (54, 198, 252). The effects of methionine toxicity can be partially alleviated by including in the diet an excess of arginine and glycine (194) or guanidinoacetic acid (148) provided these compounds are not fed at too high a level (26). From the well-known relationships among these compounds in the synthesis of creatine it appears probable that the metabolism of methionine is facilitated when guanidinoacetic acid or its precursors are provided and that they act, within certain limits, as detoxifying agents.

The striking signs observed in animals that have ingested excessive amounts of tyrosine indicate that this amino acid can be quite toxic. An excess not only retards growth but causes severe epidermal lesions. The histology and pathology of the characteristic eye and skin lesions have been described in detail (202) and some of the metabolic interrelationships which may be responsible for them have been discussed by Martin (143, 144, 145). There is no evidence that these effects can be reversed. However, they do not occur in pyridoxine-deficient rats; and, if the level of tyrosine is not too high, animals receiving an adequate diet may recover. Martin attributed the toxicity to excessive tyramine formation, but direct evidence for this has not been presented.

The growth of animals (rats and chicks have been used in most experiments) consuming excessive levels of a number of other amino acids, both essential and nonessential, may be retarded (102, 103, 198). Since there is no evidence of a common basis for these effects they have been grouped generally as amino acid toxicities. It has been suggested that the effects are inherent properties of the individual amino acids or of their breakdown products (195). The effects are often more severe when the basal diet is not adequate to support normal growth, and appear to be least severe when the diet is adequate and all of the amino acids are provided in quantities sufficient to meet the requirements.

The effects of excessive quantities of individual amino acids on the ability of the cell to concentrate other amino acids has been discussed by Christensen (46). It is quite possible that excessive levels of individual amino acids may exert adverse effects because they inhibit entry of other amino acids from the extracellular fluid into the cell.

It is unlikely that levels of individual amino acids sufficiently high to cause toxic effects would ordinarily be encountered. Nevertheless, it should be more generally realized that, relative to the requirement, the margin of safety is smaller than it is with most other dietary constituents, adverse effects being encountered in many instances in animals ingesting from two to ten times the requirement.

The question of the toxicity of the D-isomers of the amino acids is frequently raised;

however, there appears to be little evidence that the D-isomers are especially toxic (169). In fact, the D-isomers of methionine and leucine appear to be better tolerated in excess than the L-isomers. D-serine may be an exception since high levels of D-serine have been shown to cause adverse reactions (73a). There is evidence that if the diet contains a large amount of D-amino acids the rates of conversion, of those that can be used by the body, into the L-form are considerably reduced. The tolerance for high levels of D-amino acids needs further study.

Amino Acid Antagonism

An antagonist is considered to compete with the analogous dietary essential for a site of metabolic activity, thus preventing the normal reaction from going to completion.

The classical concept of metabolic antagonists arose primarily from studies on the nutrition of microorganisms and, for the most part, from studies on the effects of compounds that were not normal dietary constituents (251). The addition of structural analogues of vitamins to the medium or the diet inhibited growth and it was found necessary to include a larger amount of the vitamin itself in order to prevent the inhibition. Similar effects were observed with amino acid antagonists and, in microorganisms, antagonisms among the naturally occurring amino acids were encountered.

Antagonisms among certain naturally occurring amino acids can also be demonstrated in laboratory animals (33, 103). The only case in which there is sufficient evidence to postulate a true antagonism involves the structurally similar leucine, isoleucine, and valine. In this case, as with amino acid toxicities, the effect is most readily demonstrated by feeding a large excess of the antagonist to rats consuming a diet that is low in protein. From 1.5 to 3.0 per cent of L-leucine added to a diet containing only 9 per cent of casein caused a retardation of growth. In these experiments threonine, and possibly other amino acids, were more limiting for growth than was isoleucine. However, the growth depression caused by leucine was not alleviated by providing higher quantities of the most limiting amino acids but only when the structurally similar isoleucine was provided (103). This serves to distinguish between an amino acid antagonism and an amino acid imbalance; in the latter, a supplement of the most limiting amino acid prevents the growth depression. By carefully adjusting the levels of all of the amino acids in the diet it has been possible to demonstrate antagonisms involving leucine, isoleucine, and valine (33).

How widespread this phenomenon is and whether it may be of some practical significance is not known. There is some evidence that other amino acids such as phenylalanine may cause antagonisms (34). There is a suggestion, from some studies in which the addition of a relatively low level of leucine to a rice diet caused an increased need for isoleucine (58), that antagonisms may be demonstrated under conditions that do not involve excessive levels of amino acids. There is some reason to believe, although it is by no means proven, that the great excess of leucine in diets made up largely of corn or the corn protein, zein, may increase the need for isoleucine (32, 199). It should be noted, however, that if the level of leucine in an experimental diet is not too high the animal adapts to the diet and, after a period of severe growth retardation, begins to gain weight at a reasonable rate. Also, if the isoleucine level in the diet is just slightly above the requirement, an excess of leucine is quite well tolerated.

Further investigation will be required to determine whether some of the toxic effects that have been found to result from excessive intakes of other amino acids may also be the result of amino acid antagonisms.

Amino Acid-Vitamin Relationships

An excessive intake of an amino acid may retard the growth of experimental animals, particularly rats and chicks, raised on vitamin-deficient diets (102, 103, 198). Such effects have been observed mainly in animals fed on diets deficient in niacin, pyridoxine, and vitamin B_{12}, vitamins that are involved

in the metabolism of amino acids. Again there have been relatively few methodical studies in which a wide range of levels of both the amino acids and the vitamins have been tested, so it is not possible to draw conclusions regarding the quantitative aspects of these relationships.

A good example is the effect of methionine in increasing the severity of a deficiency of pyridoxine (54). Using a diet containing 18 per cent of casein but no pyridoxine, the addition of as little as 0.4 per cent of methionine to the diet caused a growth retardation. As the pyridoxine level of the diet was increased, more methionine was required to retard growth. However, when the methionine addition was as high as 1.5 per cent or more of the diet, growth was retarded even when the pyridoxine level was increased many fold. From this it is clear that an adverse effect may be caused by a considerably smaller quantity of an amino acid if the diet is deficient in certain vitamins. When the amino acid is in great excess, however, the adverse effect is no longer prevented by increasing the intake of the limiting vitamin.

Although it has not been adequately investigated, there is some evidence that not all amino acids fed at high levels exert the same effect on vitamin requirements. Certain vitamin requirements are increased when the overall level of protein in the diet is increased (125), but this may not be a result of the higher total amino acid intake but only of the ingestion of an excess of certain specific amino acids. These may cause an increase in the rate of catabolism of specific vitamins. To what extent an excessive intake of amino acids, generally, affects the catabolism of a vitamin, such as pyridoxine, remains to be determined.

Amino Acid Balance and Imbalance

Amino Acid Balance

The realization that the indispensable amino acids were required by the body in certain definite proportions gave rise to the concept of amino acid balance, a concept that is extremely useful for estimating the nutritive value of a protein from a knowledge of its amino acid composition (See sections 1 and 2). Proteins that provide the indispensable amino acids in roughly the proportions in which they are required by the body are said to be nutritionally well balanced. They are used very efficiently and are, therefore, required in relatively small quantities to satisfy the amino acid requirements of an animal.

Proteins that are low in one or more of the indispensable amino acids, i.e., proteins in which the amino acid proportions deviate appreciably from the proportional requirements, are said to be poorly balanced or unbalanced. There are many degrees of unbalance, ranging from proteins such as casein, which is somewhat low in the sulfur-containing amino acids, to gelatin and zein which completely lack one of the indispensable amino acids. The feeding of diets containing only unbalanced proteins can cause the development of amino acid deficiencies but, if the proteins are not too greatly out of balance, an increased intake will compensate for the deficiency and will permit maximum performance of the animal. However, under such conditions, a relative excess of all of the other amino acids will be ingested and the efficiency of utilization of the protein will be low because the extra amino acids cannot be used for protein synthesis but only as a source of energy.

On the other hand, a protein may be so severely unbalanced that no matter how much of it is consumed, the amino acid requirement will be satisfied only by supplementation with the deficient amino acid. This is certainly true of proteins, such as zein, which are completely deficient in one or more amino acids. In other cases, however, with proteins that are not completely deficient in an amino acid, although the subject may be able to consume enough so the requirement for the limiting amino acid is theoretically satisfied, the amino acid proportions may deviate so greatly from the ideal that the efficiency of utilization falls below that expected from a knowledge of the amino acid composition of the protein. Such a stage seems to

be reached when the Biological Value of a protein falls below about 60 or, roughly speaking, when the proportion of one of the indispensable amino acids is less than 60 per cent of the ideal value. Wheat gluten appears to be an example of such a protein, for when it is fed in amounts that provide the accepted requirement of the rat for lysine, optimum performance is not obtained unless still more lysine is provided either as a supplement or by increasing the protein level of the diet still more (159).

The end result in this case is analogous to that observed as a result of amino acid imbalance as defined earlier. Amino acid imbalances have usually been encountered, however, when a diet that is low in protein has been supplemented with amino acids other than the one that is most limiting for growth. This results in a retardation of growth which is prevented by a supplement of the most limiting amino acid. In both of these conditions, then, the overall effect is an increased need for the most limiting amino acid. As yet we do not know at what point there is a transition from a simple uncomplicated amino acid deficiency to an amino acid imbalance. It undoubtedly depends upon many factors, not the least of which is the level of protein intake (106). Regardless of this, it is evident that, although a comparison of the relative proportions of the amino acids in a protein with the proportional requirements of the subject may give a reasonable measure of the efficiency of utilization of a protein, the estimates obtained by such a procedure may be high for proteins that are severely unbalanced.

Amino Acid Imbalance

A measure of confusion can be avoided if the term "unbalanced" is used to indicate that the relative proportions of the amino acids in a protein deviate appreciably from the ideal and if the term "amino acid imbalance" is restricted to situations in which *the need for the most limiting amino acid is increased* because of a disproportion in the amounts of the various amino acids in the diet.

There are a number of review articles (64, 65, 67, 68, 100, 102, 197) in which the effects of imbalance on growth, on the deposition of liver fat, and on the activities of certain liver enzymes have been described.

Growth. For the most part, the effects of amino acid imbalances have been detected by their effects on growth. Perhaps the best known and most delicate of these is the growth-retarding action of less than 0.1 per cent of L-threonine in rats fed on diets low in protein (9 per cent casein) and deficient in niacin and tryptophan (98, 210). The growth retardation can readily be prevented by a small supplement of tryptophan. This case is somewhat complicated by the relationship between the vitamin, niacin, and the amino acid, tryptophan. In other instances, for example, using rice diets (58, 168), a series of amino acid imbalances have been observed in which there is no complication from a simultaneous vitamin deficiency.

As was mentioned above, the exact quantitative requirements for the production of an imbalance have not been determined. It is possible, however, almost without exception, to cause a severe depression in the rate of growth of rats fed on diets low in protein by adding to this diet a mixture containing relatively small quantities of all of the amino acids except the one that is already somewhat limiting (57, 58, 106, 168). One can envision from this situation that the supplement may stimulate the catabolism of all amino acids with a consequent loss of some of the one that is already limiting, thus causing a reduction in the growth rate (57, 196, 200).

Although young growing rats have been used in most of the studies of amino acid imbalance, similar effects can be demonstrated in mature rats (131). Also imbalances have been demonstrated in a variety of different species including mice, dogs, chickens, and pigs.

Liver fat deposition. Amino acid deficiencies (101, 208) and imbalances (104) may cause, not only reduced growth, but also an accumulation of liver fat, particularly in the periportal areas of the liver lobule (35). In general this effect is cor-

rected by amino acid or protein supplements, but in at least one case it is induced by an amino acid imbalance and in others it occurs only when the diet is supplemented to a certain level with the limiting amino acids (208). The case which appears to involve an amino acid imbalance was observed in rats fed on diets containing 9 per cent of casein as the source of protein. Rats receiving this basal diet show no abnormal accumulation of liver fat. However, a small supplement of methionine leads to fatty infiltration of the liver which can be prevented by a further supplement of threonine (104). The significance of the accumulation of liver fat cannot at present be evaluated but, since it is a common sign in severe malnutrition and may precede liver damage, it must be considered as an abnormality requiring further study.

Liver enzymes. The effects of dietary alterations on the activity of enzymes has recently been reviewed (127). In general, protein or specific amino acid deficiences are associated with decreases in the activity of a number of liver enzymes. Like the observations on liver fat deposition, adverse effects on the activity of certain liver enzymes may be detected when amino acid imbalances are induced (23, 24, 105). These observations have shown that processes such as the oxidation and decarboxylation of endogenous substrates, the oxidation of xanthine and tyrosine, and the level of pyridine nucleotides may be altered by altering relatively slightly the amino acid composition of a low-protein diet. Such changes suggest that basic metabolic processes, quite apart from the more general changes in growth and liver fat deposition, are affected by changes in the dietary proportions of amino acids.

The studies so far relating the effects of amino acid balance to enzyme changes in tissues can be considered only as preliminary in nature to further investigations. These procedures may provide a sensitive method for the detection of adverse effects from changes of amino acid proportions in a diet (173) and may provide explanations for the growth effects discussed earlier.

The significance of amino acid imbalance in human nutrition cannot yet be appraised. There have been two reports in which adverse effects, seen as a decrease in nitrogen retention, have been observed to follow the administration of amino acid supplements. One of these was in a study with adult men consuming largely rice (118), the other in children consuming diets in which the protein was provided from plant sources (205). There is, therefore, no reason to assume that the effects of amino acid imbalance are unlikely to occur in man, only that the possibilities remain unexplored.

In summary, it is evident that a variety of adverse effects from dietary additions of amino acids have been demonstrated experimentally. It is also evident, however, that our knowledge of the basis for these effects and of the quantitative aspects of amino acid imbalance is scanty. In order to appraise the significance of these adverse effects from a practical viewpoint it is necessary to continue and to expand the rather limited studies that have so far been described. The possible relationships between these observations and studies on amino acid supplementation of foods and feeds are discussed in a subsequent section.

Protein and Amino Acid Content of Diets in the United States

The previous sections have dealt with the basic concepts of protein nutrition, the methods of evaluation of protein requirements, the effects of protein deficiences, and amino acid disproportionalities. This section will indicate the status of protein and amino acids in diets prevalent in the United States as related to the principles developed previously.

Protein in U. S. Food Supply

The kinds and quantities of foods in the civilian food supply in 1957 provided about 96 gm of protein per capita per day, along with some 3,200 calories (3). These estimates were obtained by applying average food composition values to the per capita quantities of individual foods available for consumption at the retail stage of distribution.

Over the last 45 years there has been a gradual increase in the proportion of protein from animal sources, from about half in 1909-13 to two-thirds in 1957. This shift is related to an increase in consumption of dairy products, eggs, and to some extent meat, with a decrease in consumption of grain products. The total protein in the per capita food supply and the contribution of major food groups to the total for selected years is shown in table 7.

Protein in Family Diets

The nationwide study (4) of family food supplies in 1955 provided information on the kinds, quantities, and nutritive value of foods used in some 6,000 households during a week. This was the most comprehensive study made since 1936, permitting analysis by region, degree of urbanization

Table 7

Sources of Protein in U.S. Food Supply (Selected Years)

Foods	Percentage of protein from specified sources				
	1909-13	1925-29	1935-39	1947-49	1957
Dairy products	17.7	20.0	22.2	24.8	25.4
Eggs	5.2	6.1	5.7	7.0	6.6
Meat, poultry, fish, fats, oils	29.0	28.4	28.2	31.5	35.0
Grain products	35.7	31.6	28.6	23.0	19.5
Legumes, nuts	4.5	5.1	6.1	5.3	5.5
Other vegetable sources	7.9	8.8	9.2	8.4	8.0
Total	100.0	100.0	100.0	100.0	100.0
Total animal sources	51.9	54.5	56.1	63.3	67.0
Total vegetable sources	48.1	45.5	43.9	36.7	33.0
Protein per person per day—grams	102	95	91	95	96

Source: Supplements for 1956 and 1957 to Consumption of Food in the United States 1909-52, Agriculture Handbook 62, U. S. Dept. of Agriculture. (3)

(urban, rural nonfarm, farm), and income class.

For the country as a whole the average diet provided 103 gm protein per person per day, or 121 gm per nutrition unit (adult male equivalent) (4). The nutrition unit is useful for comparing households differing in size and composition. It expresses the needs of household members for nutrients in relation to the NAS—NRC recommended allowances (82) for a reference man. As in the national food supply, two-thirds of the protein in the household's food came from animal sources, with contributions from other food groups similar to those for 1957 in table 7.

Wide variation in the protein content of diets was found among households in the country as a whole as well as in each income class and in different regions (4). One per cent of the total group had food furnishing less than 50 gm of protein per nutrition unit per day, whereas 72 per cent had more than 100 gm (table 8). A similar situation was found in each region and in each urbanization group. Low-protein diets were found most frequently among families with low incomes. Even when incomes for the year were under $2,000, however, half or more of the families in each region had food that provided more than 100 gm of protein per day per nutrition unit.

These figures suggest that food supplies of most households contain generous amounts of protein. In fact, only eight per cent of the family diets failed to measure up to the NAS—NRC recommended allowances.

Protein in Diets of Individuals

Studies of diets of individuals also show wide variation in levels of nutrients among the groups studied. Some of this spread in values reflects the fact that many of the data are from one-day diet records. It is well known that in any distribution of families or individuals by level of nutrient intake there will be more cases in the extremes when the data are based on one to two days than when based on seven days or longer. In other words, there will appear to be more inadequate diets when the shorter period is used. Considerable day-to-day variation in eating occurs, and hence also in the nutritive value of the diet. It cannot be assumed that individuals whose diets were low in a nutrient on the particular day of the study would also be in the low group on another day. These points should be kept in mind in interpreting data on diets of individuals.

Table 8

Distribution of Households by Protein Content of Food Supplies per Nutrition Unit per Day (Selected Groups)

Group, income class	Number of Households	Under 50 gm	50-74 gm	75-99 gm	100-149 gm	150 gm and over
		%	%	%	%	%
All U.S., all incomes [1]	4,556	1	7	20	48	24
1-person households	369	3	8	16	38	35
Households of 2 or more persons:						
Under $2,000	630	4	14	25	37	19
$4,000-$4,999	724	*	3	21	52	24
$6,000-$7,999	456	0	4	18	54	25

Source: Dietary Levels of Households in the U.S., Report 6, Household Food Consumption Survey, 1955. U. S. Dept. of Agriculture. (4)

* Less than 0.5 per cent.

[1] Weighted sample, see source.

There is evidence, however, that in spite f generous protein supplies in the nation's ouseholds, family members do not share in ese supplies in accordance with their nutri-onal needs. Whether because of food pref-rences, notions about food needs, or other easons, diets of many individuals provide ss than recommended amounts of protein, least during the period studied.

Some groups in the population, in par-cular, fare less well than others. Women, or example, are found more often than men have poor diets compared with dietary ecommendations (47, 234, 235, 246). lder people with decreasing food intakes e likely to have diets lower in protein and her nutrients than younger adults. In a udy of one-day diets of over 1,000 Iowa omen the proportion of diets furnishing ss than 50 gm increased from 26 per cent or the 30-39 decade to 64 per cent for those 0 years and over (226).

Much information about diets of children nd adolescents has come from regional udies of diet and nutritional status (70, 1, 233, 235, 246).

Although protein is usually better sup-lied than other nutrients, many diets do ot provide recommended amounts. Usually ne diets of younger children are relatively etter than those of older children, and in eneral boys do better than girls. Diets of dolescent girls especially are likely to be ow in protein and other nutrients, com-ared with recommendations.

These and other studies suggest that food noices of many individuals fail to provide, ay-by-day, recommended amounts of pro-in. In these studies NAS—NRC's recom-ended dietary allowances are used as a oint of reference for describing dietary vels and for comparing diets of individuals ith differing nutritional needs. The pro-in allowances are believed to provide gen-rously for the needs of most individuals. does not follow necessarily that intakes low recommended amounts are inade-uate.

Whether or not protein requirements are eing met depends not only on total nitro-en intake in relation to body size, but upon the assortment and amounts of amino acids in the food, meal-by-meal, upon total calories, the level of other components in the diet, and possibly other factors. With improved methods of amino acid determination it is becoming possible to establish more precisely human requirements for indispensable amino acids, and to prepare tables of amino acid content of foods for use in dietary calculations.

Calculations of amino acids in diets need to be interpreted in the light of some standard in order to assess dietary adequacy and to show where improvement is needed. As one approach, the quantities of each amino acid may be compared with estimates of minimum requirements determined directly as in table 1 or as calculated from protein intake to maintain balance, table 3. Another approach is based on the proportionality pattern of amino acids in diets as related to a selected reference pattern. The dietary patterns may be compared with those in good food proteins such as egg or milk, or with a pattern based on minimal requirements such as that presented in table 5. A standard is needed, however, that takes into account both the quantity and assortment of indispensable amino acids in the diet.

Amino Acids in U. S. Food Supply

As background for considering the amino acid content of diets in this country, the essential amino acids available from the national food supply for a recent year were computed using average values from amino acid tables of Orr and Watt (164). The quantities of each of eight indispensable amino acids and of total sulfur-containing acids are shown in table 9 along with adult requirements from table 5. For comparison with the pattern of requirements, the amounts of amino acids in the food supply are expressed also in relation to 250 mg of tryptophan. Figures in column 2 show that the food supply provides generous amounts of these amino acids. This would be expected in a diet providing 96 gm of protein per person, two-thirds of which is from animal sources. Compared with the require-

ment pattern, the food supply contains considerably more leucine, lysine, threonine, and valine, but slightly less of the sulfur-containing acids.

Amino Acids in Diets of Individuals

It did not seem necessary to calculate the amino acid content of average family diets that provide such high levels of protein as were found in the 1955 study of household food consumption, with so much of it from animal sources. More useful information can come from self-selected diets of individuals, especially where meal-by-meal data are available.

Examples are presented in which the amino acid content of one day's meals has been estimated. The first, a diet of a middle-aged man weighing 70 kg, was chosen for calculation because it typifies the high level of protein of the average per capita diet in the 1955 survey. Moreover, like the average diet, a large share of the protein is from animal sources. The day's meals provided 106 gm of protein, three-fourths of which came from animal sources, and 2,440 calories. Of the total protein, 19 per cent was in the breakfast, 55 per cent in the noon meal, and 26 per cent in the evening meal. The amounts of indispensable amino acids provided by this diet are shown as follows:

	gm		gm
Isoleucine	5.5	Phenylalanine	4.6
Leucine	7.9	Threonine	4.6
Lysine	7.5	Tryptophan	1.3
Methionine	2.5	Valine	5.8
Cystine	1.5		
Total sulfur A.A.	4.0		

Although based on a single day's meals, these values are about the same or slightly higher than the amounts provided by the per capita food supply (table 9).

Another illustration (table 10) was taken from a study of homemakers' diets in 1948, since studies have shown homemakers to be one of the groups likely to have poor diets

Table 9

Amino Acids in U.S. Food Supply and a Comparison with Adult Requirement Pattern

Amino Acid	Amount in per capita food supply, 1953 [1]		Amino acid pattern in food supply [2]	Calculated adult male requirement [3]
	Total per day	Per gm N	(Tryptophan = 250 mg)	
(1)	(2)	(3)	(4)	(5)
	mg	mg	mg	mg
Isoleucine	5,200	340	1,080	700
Leucine	8,000	525	1,670	1,050
Lysine	6,100	400	1,270	800
Methionine	2,000	135	415	(325)
Total sulfur-containing acids	3,400	220	710	950
Phenylalanine	4,600	300	960	(325)
Tyrosine	3,600	240	750	—
Total aromatic	8,200	540	1,710	1,550
Threonine	3,900	260	810	500
Tryptophan	1,200	80	250	250
Valine	5,500	360	1,150	900

[1] Data computed by Household Economics Research Division, Agricultural Research Service, U. S. Department of Agriculture, based on estimates of per capita consumption (retail basis) supplied by Agricultural Marketing Service, U. S. Department of Agriculture.

[2] Values in column (2) multiplied by 250/1200.

[3] From table 5.

47). Diets selected were poor in many respects by present-day standards. They represent a comparatively low level of protein intake, 40 gm, about two-thirds of the recommended allowance for women. The amounts of indispensable amino acids in one-day diets of five homemakers in Birmingham and five in Minneapolis are given to show what may be expected at this level of protein intake. These diets provided at least the minimum adult female requirements (table 1) but with less margin for tryptophan and the sulfur-containing acids than in the other amino acids. Lowest levels occurred in diets 4 and 5. These were essentially cereal-bean diets with no meat except salt pork and no milk or eggs except as minor ingredients in cornbread and biscuit.

For the other six essential amino acids, quantities in the ten diets were well above any levels so far proposed as requirements. For example, the lysine content ranged from 2 to 3 gm per day. The minimal quantity given in table 1 for women is 0.50 gm (500 mg).

Amino Acid Patterns

Patterns of essential amino acids found in these diets are shown along with that found in the man's diet described earlier (table 11). Patterns are expressed in relation to tryptophan. For comparison, the patterns found in egg and milk proteins, often used as a standard, are given, as well as the pattern of amino acid requirements of adults presented in table 5.

A few observations may be noted. For example, only the ratios of the sulfur-containing amino acids to tryptophan are slightly lower than in egg protein or in the suggested requirement pattern. They compare favorably with the ratios in milk. Among the patterns of homemakers' diets, Nos. 4 and 5 are most out of line with the others, reflecting the lower tryptophan content of these

Table 10

Homemakers' Diets: Calories, Niacin and Amino Acid Content of Selected One-Day Diets Providing 40 gm of Protein [1]

Diet Number	Subject Weight	Calories	Niacin	Isoleucine	Leucine	Lysine	Methionine	Cystine	Total Sulfur A.A.	Phenylalanine [2]	Threonine	Tryptophan	Valine
	kg	no	mg	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm
Birmingham													
1	49	1,280	8.5	2.2	3.2	2.3	0.9	0.6	1.5	2.0	1.6	0.5	2.3
2	64	1,120	11.2	2.1	3.3	2.9	.9	.6	1.5	1.8	1.7	.4	2.2
3	65	2,325	11.1	2.0	3.3	2.0	.8	.8	1.5	2.2	1.6	.5	2.4
4	55	2,325	7.1	1.8	3.5	2.5	.6	.6	1.2	2.0	1.5	.3	2.2
5	55	2,500	7.2	2.2	3.7	2.6	.6	.5	1.1	2.2	1.7	.4	2.5
Minneapolis													
6	77	1,360	9.1	2.4	3.5	3.1	1.0	.6	1.6	2.1	1.9	.6	2.6
7	50	1,540	9.0	2.2	3.4	2.7	.9	.7	1.6	2.0	1.7	.5	2.4
8	70	1,720	11.4	2.2	3.4	2.8	.9	.7	1.6	2.0	1.7	.5	2.3
9	61	1,590	6.8	2.1	3.0	2.7	.8	.6	1.4	1.7	1.6	.4	2.2
10	54	1,300	6.8	2.0	3.1	2.2	.8	.6	1.4	1.8	1.5	.5	2.2

[1] Source: Unpublished data from 1948 Study of Urban Family Food Consumption. Institute of Home Economics, U. S. Department of Agriculture.

[2] Total aromatic amino acids were not computed.

two diets. The similarity between the patterns of the Minneapolis homemakers' diets furnishing 40 gm of protein and that of the man's diet providing 106 gm of protein is of interest. It helps to show why protein quality as described by an amino acid pattern cannot be considered apart from amino acid quantity in evaluating protein adequacy.

In general the patterns in the Minneapolis diets and the man's diet resemble quite closely those of egg and milk, although leucine and lysine tend to be closer to milk than to egg. Many of the ratios in the Birmingham diets also fall close to the patterns in egg and milk, although there is more variability in this group.

Amino Acids in Meals

If, for efficient use in tissue synthesis, the essential amino acids must be present at the same time in a definite pattern or proportion, it may be important to consider separate meals as well as the total day's intake. Negative nitrogen balance has been reported (86, 150) for some women eating self-selected diets that averaged about 60 to 70 gm of protein, amounts considerably greater than the homemakers received. Unequal distribution of protein foods throughout the day was given as a possible contributing factor to the negative nitrogen balance in both studies, although the essential amino acid

Table 11

Amino Acid Patterns in One-Day Diets of Individuals, Compared with Specified Patterns (Tryptophan = 1.0)

Diet & pattern	Isoleucine	Leucine	Lysine	Methionine	Cystine	Total Sulfur A.A.	Phenylalanine [1]	Threonine	Tryptophan	Valine
Homemakers' diets [2] (40 gm protein)										
Birmingham										
1	4.4	6.5	4.7	1.8	1.3	3.1	4.0	3.1	1.0	4.6
2	4.6	7.2	6.4	1.9	1.4	3.3	4.0	3.7	1.0	4.9
3	3.9	6.4	3.9	1.5	1.4	2.9	4.2	3.1	1.0	4.6
4	5.7	11.1	7.7	2.0	1.7	3.7	6.2	4.9	1.0	6.8
5	5.5	9.3	6.6	1.5	1.3	2.8	5.5	4.4	1.0	6.3
Minneapolis										
6	4.0	5.8	5.0	1.7	1.0	2.7	3.5	3.1	1.0	4.3
7	4.5	6.9	5.5	1.8	1.4	3.2	4.1	3.5	1.0	4.8
8	4.2	6.5	5.4	1.7	1.3	3.0	3.8	3.2	1.0	4.4
9	4.7	6.9	6.1	1.8	1.3	3.1	3.9	3.7	1.0	5.0
10	4.4	6.7	4.8	1.7	1.3	3.0	4.0	3.3	1.0	4.7
Man's diet [3] (106 gm protein)	4.2	6.1	5.8	1.9	1.2	3.1	3.5	3.5	1.0	4.5
Patterns										
Egg protein	4.0	5.3	3.9	1.9	1.4	3.3	3.5	3.0	1.0	4.5
Milk protein	4.5	7.0	5.5	1.7	.6	2.4	3.4	3.3	1.0	4.9
Adult requirement (table 5)	2.8	4.2	3.2	—	—	3.8	6.2 [4]	2.0	1.0	3.6

[1] Total aromatic amino acids were not computed.
[2] From table 10.
[3] See text page (38).
[4] Total aromatic amino acids.

content of individual meals was not determined. Leverton (132) noted improved nitrogen retention in college women when the protein of the diet from foods of animal origin was redistributed among meals. These results suggest that poor balance of amino acids in meals may be especially significant in low-protein diets.

For each of the ten homemakers' diets, calculations were made of the amino acid content and patterns of each meal of the day. As might be expected, the quantities of protein and component amino acids were unevenly distributed among the day's meals. Most individuals had one larger meal and one or more smaller meals. In most cases this single meal, the largest of the three, provided at least the minimal adult female requirements for indispensable amino acids.

When the patterns of separate meals were examined, it was chiefly in the sulfur-containing amino acids that ratios were slightly lower than the pattern in egg or in the suggested requirement pattern. This "pattern shortage" of sulfur-containing amino acids showed up also in the pattern of the main meal of the middle-aged man's diet, although this meal furnished 47 gm of protein, much of it from meat. It was a characteristic also of the national per capita food supply. In all of these diets the ratio of total sulfur-containing amino acids to tryptophan exceeded the ratio in milk.

Appraisal

The diet of the middle-aged man discussed above is obviously adequate in the essential amino acids compared to any standards so far proposed. The adequacy of diets of the women is not so clear cut. Even though the essential amino acids are present in what appear to be adequate amounts and in reasonably favorable proportions, this is only presumptive evidence that protein needs are being met, especially at low levels of intake. Calorie levels and amounts of other nutrients play a role in the metabolism of protein and may influence the efficiency with which proteins are used for tissue building. Some of these homemakers' diets were relatively low in calories and in niacin compared with recommended allowances; most of them were short in four or more other nutrients. Foods which would have increased the quantity and improved the quality of the protein would have reinforced the diets in other respects also. Although it might have been useful to compare these dietary findings with information on the health status of the individuals, the study was not designed for that purpose and no information concerning the health of these women was obtained.

The data and discussion presented in this section suggest that a large portion of the population in the U. S. have diets adequate in protein quality as well as in quantity. A small proportion with lower protein intakes may, in the light of present knowledge, be considered borderline with respect to protein adequacy. In the diets examined, the sulfur-containing amino acids and tryptophan are the ones most likely to be limiting compared with estimates of requirement thus far proposed. As research provides more information about human requirements for amino acids and how they are related to other dietary factors, more precise evaluation of protein adequacy in mixed diets will be possible.

Problems of Improving Nutrition by Amino Acid Supplementation

Experimental Background

Over forty years ago Osborne and Mendel owed that the relatively low growth-proting quality of wheat gluten could be subantially improved by the addition of lysine 65). Subsequent investigations have conmed these findings and have demonstrated at supplementation of cereals such as corn, e, and wheat with indispensable amino ids changes these foodstuffs from poor ality sources of protein to sources with a ality approaching that of such animal oducts as meat, milk, and eggs. Nitrogen lance studies have shown that the addin of lysine enhances the nutritive value wheat gluten for humans.

Pecora and Hundley (168), using rat owth experiments, showed that supplentation with lysine and threonine imoved the quality of rice protein. As neither sine nor threonine alone produced an imovement, they concluded that both amino ids were equally limiting. A more recent udy (192), however, indicates that a very w supplementary level of lysine alone will prove the nutritive value of rice proteins. re (224) showed that the poor protein ality of a whole wheat ready-to-eat breakst cereal was greatly improved by the adtion of lysine and was still further imoved when both lysine and threonine were ded.

Light and Frey (139) found that white ead supplemented with lysine supported much greater rate of growth of rats than d bread made from white flour alone. here was some indication that valine was so deficient in the white bread protein. out 4 per cent of casein had to be added provide a protein quality equal to that of ead supplemented with 0.25 per cent of sine and 0.27 per cent of valine.

Commercially baked white bread containing 3 per cent of nonfat milk solids was studied by Rosenberg and Rohdenburg (193). Rat growth response was greatest when the bread was supplemented with 0.8 per cent of lysine. At the end of 25 weeks, growth was essentially the same with supplementary levels of 0.2 per cent and 0.8 per cent. The studies indicated that very little, if any, loss of lysine occurred during the baking process. This is in line with work reported by Andrews (21) showing a loss of about 10 per cent. In contrast to Light and Frey (139) Rosenberg and Rohdenburg (193) found that the only important amino acid deficiency in commercial bread was that of lysine. They suggested that the earlier observation showing a response to valine supplementation may have been a result of incomplete vitamin supplementation and might possibly have been due to lack of vitamin B_{12}.

Because the superior growth rate of rats fed whole wheat flour in place of white flour has been attributed to the higher lysine content of the whole wheat protein, the effect of supplementing white flour with this amino acid was studied by workers at the British Flour Millers Research Station (120). Flour, baked into bread without the addition of milk solids, supported optimum growth when supplemented with 0.25 per cent of L-lysine, an amount approximately double that in the unsupplemented bread. Similar results have been obtained by other investigators (63, 66, 225). Thus, there can be no question about the favorable effect from lysine supplementation of wheat flour and bread. Nevertheless, it is important to note that further improvement was obtained when other amino acids, particularly threonine, were provided.

In the U. S. few people "live by bread

alone," and the value of supplementing cereal products with lysine must be assessed in relation to the diet as a whole. Westerman, Hays, and Schoneweis (242) have studied the growth of rats fed on mixed diets composed of meat, milk, eggs, and white flour. In one test in which the flour component was supplemented with 0.32 per cent lysine, rats receiving unsupplemented flour grew as well as those receiving supplemented flour. In similar tests, when the meat component was omitted, lysine supplementation improved growth and seemed to compensate for the omission of the meat. When the meat and milk components were both omitted growth was slow even when lysine, tryptophan, and valine were added. The authors concluded that diets composed of natural foods with a high cereal content require supplementation with at least small quantities of animal protein, even when they contain additional amino acids, in order to ensure healthy animals and normal rates of growth.

Hegsted, *et al.* (113) using young women as subjects, supplemented their all-vegetable diets (cereals and vegetables) with lysine or with lysine and methionine. The amino acid additions appeared to improve the Biological Value of the dietary protein but did not reveal any need for amino acid supplementation for adult populations restricted to this type of diet. The authors suggested that the proteins in fruits and vegetables may have greater value as supplements to cereal proteins than has heretofore been considered.

Rationalization for Adding Lysine to Cereal Products

A review of the problem of heat injury to protein quality, published by the Food and Nutrition Board in 1950 (81), emphasized the destruction of lysine in heat-processed ready-to-eat cereals.

Unpublished results made available to this committee included protein quality assays of several processed cereals in which the effect of adding lysine, milk, or lysine and milk was determined. A supplement of lysine improved the protein quality but not to the same degree as a supplement of mil Lysine plus milk did not improve protei quality significantly above that observed wit milk alone.

The observation that the milling of whea to produce white flour results in a lowerin of the protein quality has focused atter tion on lysine supplementation. Results re ported by Elvehjem (65) show that of th eight essential amino acids only lysine is ap preciably decreased as a result of millin In view of the deficiency of this amino aci in wheat, the decrease is significant. Th lower amino acid content is partially com pensated for by an increase in the digesti bility (112). Thus white flour protein i more efficiently utilized than a compariso of the amino acid compositions of the prod ucts of milling would indicate.

Loss of nutrients, as in the case of vita mins and minerals in the processing o wheat, corn, and rice, was one of the fac tors which led to the enrichment progra recommended by the National Researc Council. Nutritionists and flour millers wer concerned about the substantially lower vita min content of refined cereals and were seek ing means of restoring these nutrients. Thi was accomplished when the pure vitamin became commercially available. However, i was not the principle of restoration whic led to the adoption of an enrichment pro gram. Rather it was the evidence suggestin a dietary need for additional amounts o vitamins in the average American diet an the recognition that flour would be an effec tive vehicle of distribution. This reflects th continuing policy of the National Academ of Sciences—National Research Council an the Council on Foods and Nutrition of th American Medical Association in dealin with the addition of specific nutrients t foods.

Now that lysine is commercially availabl and it has been established that cereal pro teins, particularly those in refined cereals are deficient in certain amino acids as com pared with animal proteins, it is appropriat to examine the possible merit of such a ste as the fortification of flour with lysine. Flo din (74-76) has reviewed this subject ex

nsively, pointing out the advantages which ght accrue. Improvement in the utilizan of cereal proteins could be expected, rticularly in diets which contain small nounts of high-quality proteins. However, der present conditions it is not anticipated at the addition of lysine would improve rmal diets in the U. S. A. This is evident m the results of studies of the individual nino acid requirements.

Rose, *et al.* (184) have shown that the sine requirement of young adult men is w and is more than adequately met in the erage American diet. Jones, Baumann, d Reynolds (124) confirmed these obrvations in studies on women. In a review the amino acid requirements of adults, eynolds (175) expressed the belief that the ea of fortifying foods with amino acids, nile intriguing, is neither wise nor necessary this time. Clark, *et al.* (48) showed that ets in which the protein is derived almost ntirely from white flour and corn meal in mounts corresponding to today's per capita nsumption in the U. S. come close to eeting minimum adult requirements for sine. Doubling the amount of flour and rn meal would more than meet the high-t requirement. Also the inclusion of small mounts of meat, milk, or eggs would pro-de enough lysine for the normal man or oman. These workers see only a remote ssibility that diets consumed by the adult pulation in this country are inadequate in sine.

Widdowson and McCance (244) have udied undernourished children fed diets ased largely on bread. Although the diets ntained only small amounts of meat, milk, nd vegetable proteins, they enabled the nildren to achieve good nutritional status nd to thrive normally. The fact that supplentation with larger quantities of milk proced no dietary improvement suggests that sine supplementation would likewise have ad little value.

The amino acid content of diets in the nited States has been estimated in the preeding section for the national food supply nd for individual diets. In general the dietry protein provided adequate amounts and proportions of the indispensable amino acids. In some borderline cases of homemakers' diets, other nutrients as well as protein were borderline and the diets would not be corrected by lysine supplementation of cereals.

Problem of Amino Acid Balance

Amino acid balance, discussed earlier, looms large in any appraisal of the merits of amino acid fortification of foods. It is therefore referred to again.

During the early development of nutrition as a science, before the nutritional importance of the individual amino acids was realized, it was erroneously assumed that total protein intake was all important. Later, when certain amino acids were found to be indispensable nutrients, emphasis shifted toward the importance of the proportions of amino acids in the diet (191). It is just as erroneous, however, to overemphasize amino acid proportions as it is to overemphasize total protein intake. When amino acid supplementation or fortification is under consideration, both the quantitative aspects of protein intake and the proportions of the various amino acids in the protein mixture must be considered.

The concept of amino acid balance is extremely useful in nutrition. It can provide a basis for estimating the Biological Value of a protein from a knowledge of the proportions of the indispensable amino acids it contains. This is done by comparing these proportions with those of a protein of known high Biological Value or with the proportions of the amino acid requirements of the experimental subject (37, 167). Bar graphs can be used to portray vividly the proportions of amino acids present in different proteins (74), but unless the quantitative relationships are well understood, they may suggest that the amino acid requirements of a subject cannot be met by a protein which deviates from the ideal or that it is advantageous to supplement all individual food proteins to approach the ideal.

Actually, even when the quantitative basis is sound, the significance of idealized proportionality patterns of the indispensable

amino acids is only that they provide a means of estimating the efficiency with which a protein is used in the animal body (76, 80). For example, if the amino acid proportions of a hypothetical food protein deviate from the ideal pattern in that the amount of one indispensable amino acid, say threonine, is just two-thirds of that in the ideal pattern, then (assuming that the protein is highly digestible and that the deviation from the ideal pattern is not sufficiently great to cause an imbalance), in order to satisfy the requirement of the subject for threonine, the intake of the poorly balanced protein would have to be 1.5 times that of a protein having the ideal amino acid balance. If, as seems probable, 25 gm of an ideally balanced protein is sufficient to meet the amino acid requirements of the adult human (see section on Amino Acid and Protein Requirements) then these requirements should be met by 37.5 gm of a protein showing a deficit of one-third for one or more indispensable amino acids. Even assuming that some allowance above this must be made to prevent the possibility of the poor balance causing a reduction in the efficiency with which the protein is used in the body (see section on Effects of Disproportionate Amounts of Amino Acids), the amount needed to meet the requirements would still be well below the estimated per capita consumption for the United States (see section on Protein and Amino Acid Content of Diets in the U. S.). If the diet contained some well-balanced protein as well as the poorly balanced one, the total amount of protein required would be less.

The importance of expressing amino acid proportions in concrete numbers such as mg of amino acid per gm of protein nitrogen (80) rather than as the abstract numbers of a proportion cannot be overemphasized. For example, supplementation of wheat proteins with lysine brings the amino acid proportions of whole wheat almost into line with those of whole eggs; however, wheat proteins contain about one-third less of the indispensable amino acids than whole egg proteins. Therefore, when both are fed at equal levels, whole wheat proteins supplemente with lysine are still inferior despite their ex cellent amino acid balance (22, 31).

The concept that all of the essential amin acids must be present at once at the site o protein synthesis (13) has given rise to th idea that all meals and all foods should pro vide well-balanced proteins to improve th efficiency of utilization of the protein con sumed at each meal. If the protein con sumed in a particular meal were severely un balanced, any procedure that improved bal ance would diminish the percentage of amino acids that could serve only as a source o calories and would increase the percentage that could be used for protein synthesis. An improvement in the amino acid balance would be valuable if the total amino acid intake were so low and so poorly distributed that the amino acid requirements could not be satisfied.

Another point of some significance is the state of repletion of the labile proteins in the body. The dynamic state of body constituents has been emphasized (13), and although the turnover generally is not so great as is sometimes implied, certain proteins, such as liver proteins, intestinal mucosa proteins, and some others, turn over very rapidly (40, 232). If the labile proteins are fully repleted, the conservation of amino acids from these rapidly metabolized proteins would probably, over a short time, help to offset the effects of a meal containing unbalanced protein. The excess of amino acids from the unbalanced protein would provide nitrogen for dispensable amino acids and for certain functions other than protein synthesis, thus helping to prevent any severe depletion. Further, at least insofar as the adult is concerned, there is no reason to believe that some nitrogen loss for a short time is detrimental provided the overall protein intake is sufficient to maintain nitrogen equilibrium and an adequate state of protein repletion over a long period of time.

In the young growing subject, in the protein-depleted adult, and in the pregnant or lactating female, the protein requirement per unit of body weight is greater than that for maintenance. It is under these circumstances,

nen the need for protein is highest, that otein or amino acid supplements are most ely to be of benefit. Again, however, the ake required is determined by the Biogical Value of the protein consumed. If e amino acid requirements for growth or otein repletion, for pregnancy, or for lacion, are being met and the intake is sufient to maintain or replete the labile pons of the body, additional dietary protein nether it be balanced or unbalanced is necessary.

The results of animal experiments indite that amino acid imbalances are most adily demonstrated in young growing anils receiving an inadequate supply of pon. However, if the amino acid requirents are satisfied, a considerable amount of unbalanced protein or amino acid mixre can be tolerated (106). It is particuly important, therefore, in any amino acid pplementation program for infants conming less than their requirement of pon, and probably under any circumstances which the protein requirement is high, to ow that an amino acid supplement which ay be provided contains the amino acids at are most limiting in the diet.

Relation to General Policy of Food Fortification

That there is danger of causing adverse ects through the fortification of foods with nino acids has been suggested (62, 80). n appraisal of the observations on adverse ects of amino acids in experimental anals reveals that such effects are most sere under two sets of conditions: one, nen a considerable excess of a single amino id, two-fold the requirement or greater, is d; the other, when amino acid supplements lher than the amino acid in greatest deficit e added to diets deficient in niacin and ptophan (68). In the first case, levels of nino acids well beyond anything that could tionally be recommended as a supplement e involved; therefore, the results from such udies are not relevant to the question of nino acid fortification of foods. The second more cogent and emphasizes the importance of ensuring that the diet is adequate in other respects when amino acid fortification is being considered. When the diet is low in protein but adequate in all other components, additions of amino acids may still cause imbalances (102) but the likelihood of such effects decreases as the diet comes closer to satisfying the amino acid requirements.

The first step in any fortification program, as already set forth in the National Academy of Sciences—National Research Council policy on fortification of foods, should be to demonstrate that the compound being recommended as an additive to a food is one that is likely to produce a public health benefit. Because of the rather nonspecific effects of amino acid deficiencies (see section on Effects of Deficiency of Proteins and Amino Acids) and because of the difficulty of determining the sequence of amino acid deficiencies in a diet containing a mixture of proteins, marginal deficiencies are difficult to detect. The great variability in the choice of foods and in the food habits of a society as complex as that of the United States, adds to the problem of obtaining accurate information. As yet, there is no evidence that specific amino acid deficiencies are a problem in the United States.

Nevertheless, the question of the best procedure to follow in supplementing diets that are low in protein, i.e., diets deficient in more than one amino acid, deserves consideration. Studies of the effects of amino acid deficiencies on liver fat deposition (101) exemplify the complexity of the problem. Normally an increase in growth or an improvement in nitrogen retention in response to an amino acid supplement is taken as evidence of general improvement. In certain cases, however, it has been possible to demonstrate that, although growth has been stimulated by an amino acid supplement, the amount of fat accumulating in the liver is increased.* The increase in the amount of fat in the livers of animals receiving diets low in protein but otherwise adequate is not necessarily large, but as it is an abnormality

* J. S. Andrews. Unpublished data.

not observed in animals consuming an adequate diet it cannot be disregarded. The possibility that there may be other similar effects must be considered, and caution should be exercised in supplementing diets very low in protein, and therefore containing inadequate amounts of several amino acids, with a single amino acid, even if it be the one most limiting for growth. This problem is readily solved if the total protein intake can be raised.

In view of the foregoing considerations there appears to be little justification for any general amino acid additions to basic foodstuffs at the present time. There is no clear indication that, for example, the addition of lysine to white flour would improve the diets of the general population or significant segments thereof. Nor is there much likelihood that such an addition would increase the consumption of flour to the point where its use in the diet would reflect dietary advantages. Perhaps the day will come when population increases will require a greater dependence on cereals, but this is not the trend in the United States today. By the time such a situation prevails we should know more about the nutritional aspects of amino acid supplementation.

Nevertheless, it should be generally recognized that the protein of particular foods can be improved through amino acid fortification and that, from a nutritional viewpoint it is equally effective whether this is accomplished through supplementation with specific amino acids or with other food proteins. The fundamental problem is that of balancing cost against the benefits that can be anticipated from such a procedure. The committee has found no evidence of any general need for amino acid fortification; it is therefore not possible to evaluate the benefits relative to the cost.

When we come to special dietary considerations, the picture is perhaps somewhat different. Stare (221) has suggested that special dietary foods for the aged might be based on a mixture of cereals containing certain key amino acids in order to provide foods with a good quality of protein at low cost. Similarly, one might conceive of other products designed to meet the special requirements of pregnancy, illness, and other conditions in which dietary problems impose a need for more efficient utilization of proteins. Thus amino acid supplements may be of value in special conditions. Much remains to be learned, and the search for further knowledge should be continued.

Summary and Evaluation

This report includes concepts which are sic to an understanding of the modern re- nrch in the field of amino acid and protein quirements. One such concept suggests nt the body tissues are, in varying degrees, a dynamic state with the so-called "amino id metabolic pool." This dynamic state ows the body to adapt to different levels protein intake, an adaptation which can expressed in terms of a nitrogen balance.

The measurement of nitrogen balance, wever, does not adequately estimate pro- n requirements inasmuch as it is possible attain nitrogen equilibrium, accompanied a shift in "protein reserves," without hav- g provided for all of the tissues to the me degree. Knowledge of the significance the so-called "protein reserves" is frag- entary, but maintenance of reserves is be- ved to be desirable by optimal protein take. The effects of different protein in- kes, or other dietary influences, on the re- rves of different tissues, as well as the ysiological advantages which accrue from fferent degrees of reserve, have not been monstrated adequately. Further research d methodology, therefore, are needed to swer the controversial question of what nstitutes an optimal intake.

The results of many investigators show nsiderable uniformity in the data on mini- al requirements which are used as the base- ne for estimating practical protein require- ents. When an animal, including man, is d a nitrogen-free diet, the urinary nitrogen cretion decreases rapidly at first and ymptotically approaches a minimum. This inimum, closely correlated with basal etabolism, approximates 2 mg per basal lorie which provides a useful estimate for lculating the minimal protein need. On is basis, the obligatory nitrogen excretion of an individual with a basal metabolic rate of 1600 calories is 3.2 gm of nitrogen per day.

After adopting a basal minimum for estimation of protein requirements, a further step is correction for differences in the nutritive values of dietary proteins. Several methods have been devised for determining differences in the nutritive value of proteins. Perhaps the best known is Biological Value, which is defined as the percentage of the absorbed nitrogen retained in the body. Available data now indicate that the amounts and proportions of the indispensable amino acids in a protein are highly correlated with the Biological Value. Reasonable estimates of the Biological Value of dietary protein can be made from data on the amino acid composition by comparing the dietary amino acids with a "reference pattern" of the amino acids. A protein with a Biological Value of 100, such as egg, can replace the nitrogen lost in the urine on an equivalent nitrogen basis. Thus, an individual having a basal metabolic rate of 1600 calories per day and excreting 3.2 gm of nitrogen per day requires 3.2 gm of nitrogen $\times 6.25 = 20$ gm of protein of high quality. As the Biological Value decreases, proportionately more protein is needed. When converted to the more conventional expression of grams of protein per kilogram of body weight, the minimal requirements for adults of a protein with a Biological Value of 100 range from approximately 0.3 to 0.37 gm per kg per day. With a protein of Biological Value of 70, the amount required increases to 0.4 to 0.5 gm per kg per day. These figures are in agreement with nearly all experimental evidence on the minimal nitrogen requirements in adult man. There is, however, no agreement among investigators as to how closely these

values approach the optimum protein intake. No evidence is available that the recommended allowance value of 1 gm per kg is out of order or excessive.

In addition to correcting for differences in Biological Values, an additional ten per cent should be allowed to compensate for fecal loss. This would increase the daily protein intake of the two cases cited above to 22 and 30 gm respectively. The nutritive value, as opposed to the Biological Value, reflects the efficiency of the protein in fulfilling the overall body requirements. Thus the nutritive value is affected by both urinary and fecal losses.

If the Biological Value of protein is too low, perhaps below 60 per cent, there is evidence that the protein requirements cannot be met by the simple expedient of feeding additional amounts of the protein. Supplementation with amino acids or other proteins is then indicated to improve the amino acid balance in the diet.

The concept of Biological Value and the data on minimum amino acid requirements fail to explain experimental differences in some proteins and the effects of excesses of certain amino acids. Although the basis for many of these findings is essentially unexplored, the following points are known: (1) excesses of some amino acids are toxic; (2) the amino acid content of the diet may influence vitamin requirements; (3) some amino acids may interfere with the metabolism of others; (4) the requirements for some specific amino acids are a function of the amounts of other amino acids in the diet. If the amino acid requirements are well exceeded, a poor dietary pattern of amino acids is tolerated. These findings indicate gaps in the data on human requirements for amino acids but may explain some apparent inconsistencies between protein and amino acid needs.

Data on the protein requirements of children are not extensive. Estimates of the minimum requirement can be made by using the approach outlined above and adding an amount of protein, corrected for Biological Value, equivalent to that deposited as tissue during growth. During the early months of life the protein requirement is largely determined by growth, after which the amount of protein deposited per day as tissue in children is small relative to their maintenance needs. Estimates of the minimum protein requirement at different ages have been made.

Estimates of the quantitative requirements for the indispensable amino acids are available for several animals, adult men and women, and infants. Although different criteria have been used in both the experimental design and the evaluation of the data, two striking findings result: (a) the proportions of amino acids required are similar in all species, and (b) the requirements for the indispensable amino acids are surprisingly low. This uniformity in the pattern of amino acids required is interpreted to be a reflection of the similarities in the amino acid composition of the tissues of the different species. The significance of the estimated requirements of the specific indispensable amino acids may have been overemphasized in the past. There is a great need for studies of the indispensable amino acid requirements under other conditions inasmuch as they may be affected by the caloric intake, the total nitrogen intake, and other factors. In this report emphasis is placed on the pattern of amino acids in the diet as well as on the absolute level of each indispensable amino acid in the diet.

For the United States as a whole, data indicate that the average diet in 1955 provided 103 gm of protein per person per day, approximately two-thirds of which came from animal sources. Only eight per cent of the families failed to meet the NAS—NRC Recommended Allowances and only one per cent of the family diets provided less than 50 gm of protein per nutrition unit. Within the households, the homemakers, adolescent girls, and elderly people tended to consume the least. Few people had diets that were not well above the estimated minimal need. Similarly, the amounts of indispensable amino acids in the national dietary appeared generous. Even relatively poor diets, by common current standards of evaluation, provided rather generous amounts

the various indispensable amino acids nen compared with the estimated needs.

The symptoms, signs, and pathologic dis-ders attributable to protein or specific nino acid deficiencies in experimental ani-nls and man also have been reviewed and mmarized. Changes are produced in many sues of experimental animals by protein striction; specific diseases resulting from rtain amino acid deficiencies are well docu-ented. Unfortunately, similar experimental ta are not available on man. In human be-gs there are four important syndromes in nich evidences of protein deficiency are ominent. These are: (1) hunger edema,) kwashiorkor, (3) pellagra, and (4) nu-tional liver disease.

Selective amino acid supplementation has en repeatedly shown to provide a means increasing the Biological Value of some oteins and is common practice in animal eding. The appropriate supplementation cereal proteins, for example, results in a onounced increase in Biological Value nounting to an equivalent increase in avail-le protein. It should be emphasized, how-er, that we are concerned with the nutri-nal value of the diets consumed, not with at of isolated proteins. Desirability of nino acid supplementation of diets for hu-an beings should rest upon the following: demonstrated public health need; an as-rance of safety and effectiveness under the nditions in which the amino acids are to used; evidence that the procedure is prac-tical and competitive upon a price basis with other means of increasing the protein or amino acid content of the diet. The committee recognizes the potential offered by amino acid supplementation in human nutrition, but no convincing evidence of a need for such supplementation for the individual eating an average mixed diet in the United States has as yet been presented. The possibility exists, of course, that certain segments of the population could benefit by supplementation of certain food items in the diet. The use of amino acids and amino acid mixtures in specific disease states may offer considerable promise that should be thoroughly explored.

A small proportion of individuals in the United States may consume lesser amounts of protein than those called for by the Recommended Allowances of the Food and Nutrition Board. In such cases the diets are likely also to be poor in other respects. The evidence indicates that the greatest proportion of people consume protein considerably in excess of the Recommended Allowances. The question of whether there are any health problems from excessive protein intake remains to be investigated. While it cannot be denied that individual cases require consideration, and that knowledge in many areas is inadequate, it must be concluded upon the evidence available to date that malnutrition due to protein deficiency is not a general public health problem in the United States.

FOOD TECHNOLOGICAL RESEARCH INSTITUTE
4381
LIBRARY
1210-60

References

Adamstone, F. B., and H. Spector. "Tryptophan deficiency in the rat; histologic changes induced by forced feeding of an acid-hydrolized casein diet." *Arch. Path.* **49**:173-184 (1950).

Addis, T., D. D. Lee, W. Lew, and L. J. Poo. "Protein content of the organ and tissues at different levels of protein consumption." *J. Nutrition* **19**:199-205 (1940).

Agricultural Marketing Service. *Supplements for 1956 and 1957 to Consumption of Food in the United States 1909-52,* U. S. Dept. Agr., Agr. Handb. 62.

Agricultural Research Service and Agricultural Marketing Service. *Dietary Levels of Households in the United States, Northeast, North Central Region, South, West,* U. S. Dept. Agr., Household Food Consumption Survey 1955, Reports 6-10 (1957).

Albanese, A. A. "Protein and amino acid requirements of infants." *Pediatrics* **8**:455-462 (1951).

Albanese, A. A., ed. *Protein and Amino Acid Requirements of Mammals.* Academic Press, New York, 1950. 155 pp.

Albanese, A. A., and W. Buschke. "On cataract and certain other manifestations of tryptophane deficiency in rats." *Science* **95**: 584-586 (1942).

Albanese, A. A., R. A. Higgons, Gertrude M. Hyde, and L. Orto. "Biochemical and nutritional effects of lysine-reinforced diets." *Am. J. Clin. Nutrition* **3**:121-128 (1955).

Albanese, A. A., R. A. Higgons, Gertrude M. Hyde, and L. Orto. "Lysine and tryptophane content of proteins and their utilization for human growth." *Am. J. Clin. Nutrition* **4**: 161-168 (1956).

Albanese, A. A., *et al.* "The tryptophane requirement of the infant." *Bull. Johns Hopkins Hosp.* **80**:158-165 (1947).

Albanese, A. A., *et al.* "The isoleucine requirement of the infant." *J. Nutrition* **35**: 177-183 (1948).

Albanese, A. A., *et al.* "The sulfur amino acid requirements of the infant." *J. Nutrition* **37**:511-520 (1949).

Allison, J. B. "Biological evaluation of proteins." *Physiol. Revs.* **35**:664-700 (1955).

Allison, J. B. "Evaluation of dietary proteins." *Nutrition Revs.* **14**:129-131 (1956).

15. Allison, J. B. "Nitrogen balance and the nutritive value of proteins." *J. Am. Med. Assoc.* **164**:283-289 (1957).
16. Allison, J. B., in *Amino Acid Malnutrition.* Rutgers Univ. Press, New Brunswick, N. J., 1957.
17. Allison, J. B. "Calories and protein nutrition." *Ann. N. Y. Acad. Sci.* **69**:1009-1024 (1958).
18. Allison, J. B. "Balance and imbalance of amino acids both in regard to human and animal nutrition." *Voeding* **19**:119-138 (1958).
19. Allison, J. B. "Amino acid requirements." *Feedstuffs* Dec. 6 5-7, (1958).
20. Almquist, H. J. "Fallacy in analysis of nutritional requirement data." *Arch. Biochem.* **50**:503 (1954).
21. Andrews, J. S. "Nutritional aspects of cereal proteins," *Bakers' Digest* **27**:80-81 (1953).
22. Andrews, J. S. "Proteins and Nutrition." *Cereal Science Today* **2**:242 (1957).
23. Arata, Dorothy, Gyda Svenneby, J. N. Williams, Jr., and C. A. Elvehjem. "Metabolic factors and the development of fatty livers in partial threonine deficiency." *J. Biol. Chem.* **219**:327-333 (1956).
24. Arata, Dorothy, *et al.* "Some effects of dietary threonine, tryptophan, and choline on liver enzymes and fat." *Proc. Soc. Exptl. Biol. Med.* **87**:544-549 (1954).
25. Baptist, N. G., and Beatrice V. deMel. "Growth and amino acid intakes of children on a cereal-legume-vegetable diet." *Brit. J. Nutrition* **9**:156-170 (1955).
26. Baron, H. "Some effects of *DL*- methionine and glycocyamine on growth and nitrogen retention in rats." *J. Nutrition* **64**:229-239 (1958).
27. Beach, E. F., S. S. Bernstein, and Icie G. Macy. "Intake of amino acids by breast-milk-fed infants and amino acid composition of cow's milk and human milk." *J. Pediat.* **19**:190-200 (1941).
28. Beach, E. F. Bertha Munks, A. Robinson, and Icie G. Macy. "Dietary evaluation of animal proteins from their amino acid contents." *J. Am. Dietet. Assoc.* **19**:570-757 (1943).
29. Behar, M., *et al.* "Principles of treatment and prevention of severe protein malnutrition in

children (kwashiorkor)." *Ann. N. Y. Acad. Sci.* **69**:954-968 (1958).
30. Bender, A. E. "Relation between protein efficiency and net protein utilization." *Brit. J. Nutrition* **10**:135 (1956).
31. Bender, A. E. "Nutritive value of bread protein fortified with amino acids." *Science* **127**:874 (1958).
32. Benton, D. A., A. E. Harper, and C. A. Elvehjem. "Effect of isoleucine supplementation on the growth of rats fed zein or corn diets." *Arch. Biochem. Biophys.* **57**:13-19 (1955).
33. Benton, D. A., A. E. Harper, H. E. Spivey, and C. A. Elvehjem. "Leucine, isoleucine, and valine relationships in the rat." *Arch. Biochem. Biophys.* **60**:147-155 (1956).
34. Benton, D. A., A. E. Harper, H. E. Spivey, and C. A. Elvehjem. "Phenylalanine as an amino acid antagonist for the rat." *Arch. Biochem. Biophys.* **60**: 156-163 (1956).
35. Best, C. H., W. S. Hartroft, C. C. Lucas, and J. H. Ridout. "Effects of dietary protein, lipotropic factors, and realimentation on total hepatic lipids and their distribution." *Brit. Med. J.* **i**:1439-1444 (1955).
36. Block, R. J., and Diana Bolling. *The Amino Acid Composition of Proteins and Foods,* 2nd ed. C. C. Thomas, Springfield, Ill., 1951. 576 pp.
37. Block, R. J., and H. H. Mitchell. "The correlation of the amino acid composition of proteins with their nutritive value." *Nutrition Abstr. & Revs.* **16**:249-278 (1946-47).
38. Block, R. J., and Kathryn W. Weiss. *Amino Acid Handbook: Methods and Results of Protein Analysis.* C. C. Thomas, Springfield, Ill., 1956. 384 pp.
39. Block, R. J., *et al.* "Effects of baking and toasting on the nutritional value of proteins." *Arch. Biochem.* **10**:295-301 (1946).
40. Borsook, H. "Protein turnover and incorporation of labeled amino acids into tissue proteins in *vivo* and in *vitro*." *Physiol. Revs.* **30**:206-219 (1950).
41. Bosshardt, D. K., and R. H. Barnes. "The determination of metabolic fecal nitrogen and protein digestibility." *J. Nutrition* **31**:13-21 (1946).
42. Bricker, Mildred, H. H. Mitchell, and Gladys M. Kinsman. "The protein requirements of adult human subjects in terms of the protein contained in individual foods and food combinations." *J. Nutrition* **30**:269-283 (1945).
43. Burke, Bertha S., Virginia A. Beal, S. B. Kirkwood, and H. C. Stuart. "Nutrition studies during pregnancy." *Am. J. Obstet. Gynecol.* **46**:38-52 (1943).
44. Cannon, P. R. "The relation of protein metabolism to antibody production and resistance to infection." *Advances in Protein Chem.* **2**:135-154 (1945).
45. Cartwright, G. E., *et al.* "Anemia, hypoproteinemia, and cataracts in swine fed casein hydrolyzate or zein. Comparison with pyridoxine deficiency anemia." *J. Clin. Invest.* **24**:268-277 (1945).
46. Christensen, H. N., in *Symposium on Amino Acid Metabolism,* W. B. McElroy and H. B. Glass, eds. The Johns Hopkins Press, Baltimore, 1955.
47. Clark, F., and L. J. Fincher. *Nutritive Content of Homemakers' Meals, Four Cities, Winter 1948.* U. S. Dept. of Agr., Agr. Inf. Bull. 112, 1954. 67 pp.
48. Clark, Helen E., *et al.* "Amino acid requirements of men and women. I. Lysine." *J. Nutrition* **62**:71-82(1957).
49. Cole, W. H., ed. *Some Aspects of Amino Acid Supplementation (12th Annual Conference on Protein Metabolism).* Rutgers Univ. Press, New Brunswick, N. J., 1956. 85 pp.
50. Cole, W. H., ed. *Amino Acid Malnutrition (13th Annual Conference on Protein Metabolism).* Rutgers Univ. Press, New Brunswick, N. J., 1957. 98 pp.
51. DaCosta, Esther, and Ruth Clayton. "Histological and biochemical changes in the rat adrenal after dietary restriction and rehabilitation." *Am. J. Physiol.* **171**:717 (1952).
52. Dalgliesh, C. E. "Interrelations of tryptophan, nicotinic acid, and other B vitamins." *Brit. Med. Bull.* **12**:49-51 (1956).
53. Dean, R. F. A. "Plant proteins in child feeding." *Med. Research Council (Brit.) Spec. Rep. Ser. No.* **279**:1-163 (1953).
54. DeBey, H. J., E. E. Snell, and C. A. Baumann. "Studies on the interrelationship between methionine and vitamin B_6." *J. Nutrition* **46**:203-214 (1952).
55. Deshpande, P. D., A. E. Harper, M. Collins, and C. A. Elvehjem. "Biological availability of isoleucine." *Arch. Biochem. Biophys.* **67**: 341-349 (1957).
56. Deshpande, P. D., A. E. Harper, and C. A. Elvehjem. "Nutritional improvement of white flour with protein and amino acid supplements." *J. Nutrition* **62**:503-512 (1957).
57. Deshpande, P. D., A. E. Harper, and C. A. Elvehjem. "Amino acid imbalance on low fibrin diets." *J. Biol. Chem.* **230**:327-33 (1958).
58. Deshpande, P. D., A. E. Harper, F. Quiros Perez, and C. A. Elvehjem. "Further observations on the improvement of polished rice with protein and amino acid supplements." *J. Nutrition* **57**:415-428 (1955).
59. Dodd, K., and A. S. Minot. "Edema in infancy and childhood as an expression of chronic dietary insufficiency." *J. Pediat.* **8**: 442-458 (1936).
60. Dubos, R. J., and R. W. Schaedler. "Effect of dietary proteins and amino acids on sus

ceptibility of mice to bacterial infections." *J. Exptl. Med.* **108**:69 (1958).
Ebbs, J. H., F. F. Tisdall, and W. A. Scott. "Influence of prenatal diet on mother and child." *J. Nutrition* **22**:515-526 (1941).
Editorial. *"Amino acid imbalance and supplementation."* J. Am. Med. Assoc. **161**: 884 (1955).
Elvehjem, C. A. "Studies on amino acid supplementation of cereals." *Cereal Science Today* **1** (5):162-164 (1956).
Elvehjem, C. A. "Amino acid imbalance." *Federation Proc.* **15**:965-970 (1956).
Elvehjem, C. A. "Amino acid balance in nutrition." *J. Am. Dietet. Assoc.* **32**:305-308 (1956).
Elvehjem, C. A. "Problems in meeting amino acid requirements." *J. Am. Dietet. Assoc.* **32**:921-925 (1956).
Elvehjem, C. A., and A. E. Harper. "Importance of amino acid balance in nutrition." *J. Am. Med. Assoc.* **158**:655 (1955).
Elvehjem, C. A. and W. A. Krehl. "Dietary interrelationships and imbalance in nutrition." *Borden's Rev. Nutrition Research* **16**: 69-84 (1955).
Elvehjem, C. A., R. J. Madden, F. M. Strong, and D. W. Woolley. "The isolation and identification of the antiblacktongue factor." *J. Biol. Chem.* **123**:137-149 (1938).
Eppright, E. S., and C. Roderuck. "Diet and nutritional status of Iowa School children." *Am. J. Pub. Health* **45**:464-471 (1955).
Eppright, E. S., *et al. Nutrition of Nine-, Ten-, and Eleven-year-old Public School Children in Iowa, Kansas, and Ohio. I. Dietary findings.* Iowa Agr. Expt. Sta. Res. Bull. 434, December 1955 (North Central Regional Pub. 59). pp. 614-631.
Estramera, H. R., and W. D. Armstrong. "Effect of protein intake on the bones of mature rats." *J. Nutrition* **35**:611-618 (1948).
Ferraro, A., and L. Roizin. "Essential amino-acid deficiency, clinicopathologic findings in rats. II. Valine." *J. Neuropathol. Exptl. Neurol.* **6**:383-390 (1947).
Fishman, W. H., and C. Artom. "Variation of some constituents in urine of rats receiving DL-serine and DL-alanine." *Proc. Soc. Exptl. Biol. Med.* **60**:288-292 (1945).
Flodin, N. W. "Amino acids and proteins; their place in human nutrition problems." *J. Agr. Food Chem.* **1**:222-235 (1953).
Flodin, N. W. "The philosophy of amino acid fortification of foods." *Cereal Science Today* **1**(5):165-170 (1956).
Flodin, N. W. "Amino acid balance and efficiency of protein utilization." *Metabolism* **6**:350-364 (1957).
Folin, O. "A theory of protein metabolism." *Am. J. Physiol.* **13**:117-138 (1905).
Follis, R. H., Jr. "Nutrition and bone disease." *J. Mt. Sinai Hosp. N. Y.* **16**:1-13 (1949).
79. Follis, R. H., Jr. *Deficiency Disease.* C. C. Thomas, Springfield, Ill. 1958. 577 pp.
80. Food and Agriculture Organization of the United Nations: *Protein Requirements.* FAO Nutritional Studies No. 16. Rome, 1957. 52 pp.
81. Food and Nutrition Board. *The Problem of Heat Injury to Dietary Protein.* Reprint and Circular Series No. 131. Natl. Acad. Sci.—Natl. Research Council, Washington, 1950. 19 pp.
82. Food and Nutrition Board. *Recommended Dietary Allowances* (revised 1958). Publ. 589. Natl. Acad. Sci.—Natl. Research Council, Washington, 1958. 36 pp.
83. Food and Nutrition Board. *Inadequate Diets and Nutritional Deficiencies.* NAS—NRC Bull. 109, 1943. 56 pp.
84. Frandsen, A. M., *et al.* "The effects of various levels of dietary protein on skeletal growth and endochondral ossification in young rats." *Anat. Record* **119**:247-265 (1954).
85. Frazier, L. E., P. R. Cannon, and R. H. Hughes. "The problem of heat injury to dietary protein." *Food Research* **18**:91-103 (1953).
86. Futrell, Mary F., Ruth N. Lutz, May S. Reynolds, and C. A. Baumann. "Studies on amino acids in self-selected diets." *J. Nutrition* **46**:299-311 (1952).
87. Geiger, E., G. W. Courtney, and L. E. Geiger. *The physiological availability of some essential amino acids present in zein, in acid and in enzymatic hydrolyzate of zein."* Arch. Biochem. **41**:74-82 (1952).
88. Glynn, L. E., H. P. Himsworth, and A. Neuberger. "Pathological states due to deficiency of the sulfur-containing amino acids." *Brit. J. Exptl. Pathol.* **26**: 326-337 (1945).
89. Goldsmith, Grace A., H. P. Sarett, U. D. Register, and J. Gibbens. "Studies of niacin requirement in man; experimental pellagra in subjects on corn diets low in niacin and tryptophan." *J. Clin. Invest.* **31**:533-542 (1952).
90. Goldsmith, Grace A. "Niacin-tryptophan relationships in man and niacin requirements." *Am. J. Clin. Nutr.* **6**:479-486 (1958).
91. Gómez, F., R. Ramos-Galván, J. Cravioto, and S. Frenk. "Prevention and treatment of chronic severe infantile malnutrition (kwashiorkor)." *Ann. N. Y. Acad. Sci.* **69**:969-988, (1958).
92. Greenstein, J. P., *et al.* "Quantitative nutritional studies with water soluble, chemically defined diets I-V." *Arch. Biochem. and Biophys.* **72**:396-456 (1957).
93. Griffith, W. H., and J. F. Nyc. "Choline," in *The Vitamins: Chemistry, Physiology, Pa-*

thology. W. A. Sebrell and R. S. Harris, eds. Academic Press, New York, 1954. 3 vols.

94. Grossman, M. I., H. Greengard, and A. C. Ivy. "Effect of dietary composition on pancreatic enzymes." *Am. J. Physiol.* **138**:676-682 (1943).
95. Gullino, P., *et al.* "Studies on the metabolism of amino acids and related compounds *in vivo*. I. Toxicity of amino acids, individually and in mixtures, and the essential protective effect of L-arginine." *Arch. Biochem. Biophys.* **64**:319-332 (1956).
96. György, P., and H. Goldblatt. "Further observations on the production and prevention of dietary hepatic injury in rats." *J. Exptl. Med.* **89**:245-268 (1949).
97. Hall, H. K., L. L. Bowles, V. P. Sydenstricker, and H. L. Schmidt, Jr. "Cataracts due to deficiencies of phenylalanine and of histidine in the rat. A comparison with other types of cataracts." *J. Nutrition* **36**:277-295 (1948).
98. Hankes, L. V., L. M. Henderson, W. L. Brickson, and C. A. Elvehjem. "Effect of amino acids on growth of rats on niacin-tryptophan-deficient rations." *J. Biol. Chem.* **174**:873-881 (1948).
99. Hansen, J. D., E. E. Howe, and J. F. Brock. "Amino acids and kwashiorkor." *Lancet* **ii**: 911-913 (1956).
100. Harper, A. E. "Amino acid imbalances, toxicities, and antagonisms." *Nutrition Revs.* **14**:225-227 (1956).
101. Harper, A. E. "Nutritional fatty livers in rats." *Am. J. Clin. Nutrition* **6**:242-253 (1958).
102. Harper, A. E. "Balance and imbalance of amino acids." *Ann. N. Y. Acad. Sci.* **69**: 1025-1041 (1958).
103. Harper, A. E., D. A. Benton, and C. A. Elvehjem. "L-leucine, an isoleucine antagonist in the rat." *Arch. Biochem. Biophys.* **57**:1-12 (1955).
104. Harper, A. E., D. A. Benton, M. E. Winje, and C. A. Elvehjem. " 'Antilipotropic' effect of methionine in rats fed threonine- deficient diets containing choline." *J. Biol. Chem.* **209**: 159-163 (1954).
105. Harper, A. E., *et al.* "Effect of partial deficiency of threonine on enzyme activity and fat deposition in the liver." *Proc. Soc. Exptl. Biol. Med.* **84**:414-417 (1953).
106. Harper, A. E. "Amino acid balance and imbalance. I. Dietary level of protein and amino acid imbalance." *J. Nutrition* **68**: 405-418 (1959).
107. Harte, R. A., and J. J. Travers. "Human amino acid requirements." *Science* **105**:15-16 (1947).
108. Harvey, C. C., and M. K. Horwitt. "Excretion of essential amino acids by man on a controlled protein intake." *J. Biol. Che* **178**:953-962 (1949).
109. Hawley, E. E., J. R. Murlin, E. S. Nass and T. A. Szymanski. "Biological values six partially purified proteins." *J. Nutriti* **36**:153-169 (1948).
110. Hegsted, D. M. "Theoretical estimates the protein requirements of children." *J. A Dietet. Assoc.* **33**:225-232 (1957).
111. Hegsted, D. M., and Jane Worcester. study of the relation between protein e ciency and gain in weight on diets of consta protein content." *J. Nutrition* **33**:685-7 (1947).
112. Hegsted, D. M., *et al.* "Protein requiremer of adults." *J. Lab. Clin. Med.* **31**:261-2 (1946).
113. Hegsted, D. M., *et al.* Lysine and methioni supplementation of all-vegetable diets f human adults." *J. Nutrition* **56**:555-5 (1955).
114. Hoffman, W. S., and G. C. McNeil. "T enhancement of the nutritive value of whe gluten by supplementation with lysine, determined from nitrogen balance indices human subjects." *J. Nutrition* **38**:331-3 (1949).
115. Holt, L. E., Jr. "Nutritional conferenc Ibadan." *W. African Med. J.* **7**:38 (1958).
116. Holt, L. E., Jr., and Selma E. Snyderman, *Some Aspects of Amino Acid Supplemen tion.* Rutgers Univ. Press, New Brunswic N. J., 1956.
117. Howard, H. W., W. J. Monson, C. D. Bau and R. J. Block. The nutritive value of bre flour proteins as affected by practical supp mentation with lactalbumin, nonfat dry m solids, soy bean flour, wheat gluten a lysine." *J. Nutrition* **64**:151-165 (1957).
118. Hundley, J. M., *et al.* "Lysine, threonine a other supplements to rice diets in ma amino acid imbalance." *Am. J. Clin. Nut tion* **5**:316 (1957).
119. Hunter, H. A. "Hyproteinemia in relation the dental tissues." *J. Dental Research* **2** 73-86 (1950).
120. Hutchinson, J. B., T. Moran, and J. Pa "Effect on the growth of weanling rats supplementing the protein of white bre with L-lysine." *Nature* **178**:46-47 (1956
121. Jackson, C. M. *The Effects of Inanition a Malnutrition upon Growth and Structu* Blakiston, Philadelphia, 1925.
122. Jelliffe, D. B. *Infant Nutrition in the Su tropics and Tropics.* World Health Organiz tion Monograph Series No. 29, Geneva, 19 237 pp.
123. Jelliffe, D. B., G. Bras, and K. L. Stua "Kwashiorkor and marasmus in Jamaican i fants." *West Indian Med. J.* **3**:43-55 (1954
124. Jones, Evelyn M., C. A. Baumann, and M S. Reynolds. "Nitrogen balances of wom

maintained on various levels of lysine." *J. Nutrition* **60**:549-562 (1956).
5. Kaunitz, H., C. A. Slanetz, and R. E. Johnson. "Dietary casein level and B-factor deficiencies produced by anatogonists." Science **122**:1017-1018 (1955).
6. Keys, A., *et al. The Biology of Human Starvation.* Univ. of Minnesota Press, Minneapolis, 1950. 2 vols.
7. Knox, W. E., V. H. Auerbach, and E. C. C. Lin. "Enzymic and metabolic adaptations in animals." *Physiol. Revs.* **36**:164-254 (1956).
8. Kosterlitz, H. W. "The effects of changes in dietary protein in the composition and structure of the liver cell. *J. Physiol.* **106**:194-210 (1947).
9. Kosterlitz, H. W., and R. M. Campbell. "Storage of protein in the adult animal." *Nutrition Abstr. & Revs.* **15**:1-14 (1945).
10. Kühnau, J. "Biochemie des Nahrungsweisses." *Angew. Chem.* **61**:357-365 (1949).
11. Kumta, U. S., A. E. Harper, and C. A. Elvehjem. "Amino acid imbalance and nitrogen retention in adult rats." *J. Biol. Chem.* **233**:1505-1508 (1958).
12. Leverton, Ruth M., and Mary R. Gram. "Nitrogen excretion of women related to the distribution of animal protein in daily meals." *J. Nutrition* **39**:57-65 (1949).
13. Leverton, Ruth M., *et al.* "The quantitative amino acid requirements of young women. I. Threonine." *J. Nutrition* **58**:59-81 (1956).
14. *Ibid.*, "II. Valine," pp. 83-93.
15. *Ibid.*, "III. Tryptophan," pp. 219-229.
16. *Ibid.*, "IV. Phenlalanine, with or without tyrosine," pp. 341-353.
17. *Ibid.*, "V. Leucine," pp. 355-365.
18. Lewis, H. B. "The metabolism of sulfur. IX. The effect of repeated administration of small amounts of cystine." *J. Biol. Chem.* **65**:187-195 (1925).
19. Light, R. F., and C. N. Frey. "The nutritive value of white and whole wheat breads." *Cereal Chem.* **20**:645-660 (1943).
20. Loewenthal, L. A., and W. Montagna. "Effects of caloric restriction on skin and hair growth in mice." *J. Invest. Dermatol.* **24**: 429-433 (1955).
21. Lynch, H. D., and W. D. Snively, Jr. Hypoproteinosis of childhood." *J. Am. Med. Assoc.* **147**:115-119 (1951).
22. Madden, S. C., F. W. Anderson, J. C. Donovan, and G. H. Whipple. "Plasma protein production influenced by amino acid mixtures and lack of essential amino acids." *J. Exptl. Med.* **82**:77-92 (1945).
23. Martin, G. J. "Toxicity of tyrosine in pyridoxine-deficient rats," *J. Biol. Chem.* **166**:389 (1946); "Toxicity of amino acids as influenced by riboflavin deficiency," *Proc. Soc. Exptl. Biol. Med.* **63**:528-529 (1946); "Toxicity of histidine in normal and avitaminotic rats," *Exptl. Med. Surg.* **5**:191-195 (1947); "Toxicity of tyrosine in normal and vitamin-deficient animals," *J. Am. Pharm. Assoc.* **36**:187-190 (1947).
24. Martin, G. J. "The hypertensive effect of diets high in tyrosine." *Arch. Biochem.* **1**: 397-401 (1943).
25. Martin, G. J., and W. C. Hueper. "Biochemical lesions produced by diets high in tyrosine." *Arch. Biochem.* **1**:435-438 (1943).
26. McCance, R. A. "The history, significance, and aetiology of hunger oedema," in *Studies of Undernutrition, Wuppertal 1946-49.* Med. Research Council (Brit.) Spec. Rep. Ser. No. **275**:21-82 (1951).
27. McGanity, W. J., *et al.* "The Vanderbilt cooperative study of maternal and infant nutrition. V. Description and outcome of obstetric sample." *Am. J. Obstet. Gynecol.* **67**: 491-500 (1954).
28. McKittrick, D. S. "Interrelations of choline and methionine in growth and the action of betaine in replacing them." *Arch. Biochem.* **15**:133-155 (1947).
29. Meister, A. *Biochemistry of Amino Acids.* Academic Press, New York, 1957. 485 pp.
30. Mertz, E. T., *et al.* "Essential amino acids in self-selected diets of older women." *J. Nutrition* **46**:313-322 (1952).
31. Miller, L. L. "Changes in rat liver enzyme activity with acute inanition; relation of loss of enzyme activity to liver protein loss." *J. Biol. Chem.* **172**:113-121 (1948).
32. Miller, L. L., C. G. Bly, M. L. Watson, and W. F. Bale. "The dominant role of the liver in plasma protein synthesis." *J. Exptl. Med.* **94**:431-453 (1951).
33. Miller, D. S. and A. E. Bender. "The determination of the net utilization of proteins by a shortened method." *Brit. J. Nutrition* **9**:382 (1955).
34. Mitchell, H. H. *Minimum Protein Requirements of Cattle.* Bull. No. 67 (Comm. on Animal Nutrition). Natl. Acad. Sci.—Natl. Research Council, Washington, 1929. 84 pp.
35. Mitchell, H. H., in *Protein and Amino Acid Requirements of Mammals,* A. A. Albanese, ed. Academic Press, New York, 1950. 155 pp.
36. Mitchell, H. H. "The dependence of the biological value of food proteins upon their content of essential amino acids," in *Die Bewertung der Futterstoffe und andere Probleme der Tierernährung,* K. Nehring, ed. *Wiss. Abhandl. deut. Akad. Landwirtsch.* **5** (vol. 2):279-325 (1954).
37. Mitchell, H. H., and R. J. Block. "Some relationships between amino acid contents of proteins and their nutritive values for rats. *J. Biol. Chem.* **163**:599-620 (1946).

158. Moore, T. B., and J. E. Wilson. "Histidine as an essential nutrient for the adult rat." *J. Nutrition* **62**:357-365 (1957).
159. Munaver, S. M., and A. E. Harper. "Amino acid balance and imbalance. II. Dietary level of protein and lysine requirement." *J. Nutrition* **69**:58-64 (1959).
160. Nasset, E. S., in *Some Aspects of Amino Acid Supplementation.* Rutgers Univ. Press, New Brunswick, N. J., 1956.
161. Nasset, E. S. "Comparison of amino acid mixtures and egg protein as sources of dietary nitrogen." *J. Nutrition* **61**:555-569 (1957).
162. Nelson, W. E., ed. *Mitchell-Nelson Textbook of Pediatrics,* 5th ed. W. B. Saunders Co., Philadelphia, 1950. p. 60.
163. Nelson, Marjorie M., and H. M. Evans. "Relation of dietary protein levels to reproduction in the rat." *J. Nutrition* **51**:71-84 (1953).
164. Orr, Martha L., and Bernice K. Watt. *Amino Acid Content of Foods.* ARS, U. S. Dept. of Agr., Home Economics Research Report No. 4, Washington, 1957. 82 pp.
165. Osborne, T. B., and L. B. Mendel. "Amino acids in nutrition and growth." *J. Biol. Chem.* **17**:325-349 (1914).
166. Osborne, T. B., L. B. Mendel, and Edna L. Ferry. "A method of expressing numerically the growth-promoting value of proteins." *J. Biol. Chem.* **37**:223-229 (1919).
167. Oser, B. L. "A method for integrating essential amino acid content in nutritional evaluation of protein." *J. Am. Dietet. Assoc.* **27**:396-402 (1951).
168. Pecora, L. J., and J. M. Hundley. "Nutritional improvement of white polished rice by addition of lysine and threonine." *J. Nutrition* **44**:101-112 (1951).
169. Phillips, W. A., and C. P. Berg. "Effect upon growth of the D isomers in synthetic mixtures of the essential amino acids." *J. Nutrition* **53**:481-498 (1954).
170. Popper, H. P., and F. Schaffner. *Liver Structure and Function.* Blakiston, New York, 1957. 777 pp.
171. Pratt, E. L., *et al.* "The threonine requirement of the normal infant." *J. Nutrition* **56**: 231-251 (1955).
172. Price, J. P., and W. M. Hart. "Malnutrition and anemia in young children; clinical study of 50 cases." *J. Am. Med. Assoc.* **148**: 5-10 (1952).
173. Prigmore, J. R., J. W. Bothwell, and J. N. Williams, Jr. "Response of liver enzymes and other proteins to amino acid deficient diets." *Proc. Soc. Exptl. Biol. Med.* **88**: 43-46 (1955).
174. Rechcigl, M., Jr., J. K. Loosli, and H. H. Williams. "The net utilization of non-specific nitrogen sources for the synthesis of non-essential amino acids. I. Growth and nitrogen utilization." *J. Nutrition* **63**:177-192 (1957).
175. Reynolds, May S. "Amino acid requirements of adults." *J. Am. Dietet. Assoc.* **33**:1015-1018 (1957).
176. Reynolds, May S., Mary F. Futrell, and C. A. Baumann. "Nitrogen balances and amino acid content of self-selected diets of women." *J. Am. Dietet. Assoc.* **29**:359-364 (1953).
177. Rittenberg, D. "Dynamic aspects of the metabolism of amino acids." *Harvey Lectures, Ser.* **44**:200-219 (1948-49).
178. Rose, W. C., and S. H. Eppstein. "The dietary indispensability of valine." *J. Biol. Chem.* **127**:677-684 (1939).
179. Rose, W. C. "Amino acid requirements of man." *Federation Proc.* **8**:546-552 (1949).
180. Rose, W. C. "Half-century of amino acid investigation." *Chem. and Eng. News* **30**: 2385-2393 (1952).
181. Rose, W. C., *et al.* "The amino acid requirements of man. VI. The role of the caloric intake." *J. Biol. Chem.* **210**: 331-342 (1954).
182. *Ibid.,* "VII. General procedures; the tryptophan requirement," **211**:815-827.
183. *Ibid.,* "IX. The phenylalanine requirement," **213**:913-922 (1955).
184. *Ibid.,* "X. The lysine requirement," **214**:579-587.
185. *Ibid.,* "XI. The threonine and methionine requirements," **215**:101-110.
186. *Ibid.,* "XII. The leucine and isoleucine requirements," **216**:225-234.
187. *Ibid.,* "XIII. The sparing effect of cystine on the methionine requirement," **216**:763-773.
188. *Ibid.,* "XIV. The sparing effect of tyrosine on the phenylalanine requirement," **217**:95-101.
189. *Ibid.,* "XV. The valine requirement; summary and final observations," **217**:987-995.
190. *Ibid.,* "XVI. The role of the nitrogen intake," **217**:997-1004.
191. Rose, W. C. "The amino acid requirements of adult man." *Nutrition Abstr. and Revs.* **27**:631-647 (1957).
192. Rosenberg, H. R., and R. Culik. "The improvement of the protein quality of white rice by lysine supplementation." *J. Nutrition* **63**: 477-487 (1957).
193. Rosenberg, H. R., and E. L. Rohdenburg. "Fortification of bread with lysine; nutritional value of fortified bread." *Arch. Biochem.* **37**:461-468 (1952).
194. Roth, J. S., and J. B. Allison. "Effect of feeding excess glycine, L-arginine, and DL-methionine to rats on a casein diet." *Proc. Soc. Exptl. Biol. Med.* **70**:327-330 (1949).
195. Russell, W. C., M. W. Taylor, and J. M. Hogan. "Effect of excess essential amino acids on growth of the white rat." *Arch. Biochem. Biophys.* **39**:249-253 (1952).

Salmon, W. D. "Tryptophan requirement of rat as affected by niacin and level of dietary nitrogen." *Arch. Biochem.* **51**:30-41 (1954).
Salmon, W. D. "The significance of amino acid imbalance in nutrition." *Am. J. Clin. Nutrition* **6**:487-494 (1958).
Sassen, A. "Revue de la toxicité des acides aminés." *Acta Gastro-enterol.* Belg. **12**:944-961 (1955).
Sauberlich, H. E., W-Y. Chang, and W. D. Salmon. "The comparative nutritive value of corn of high and low protein content for growth in the rat and chick." *J. Nutrition* **51**:623-635 (1953).
Sauberlich, H. E., and W. D. Salmon. "Amino acid imbalance as related to tryptophan requirement of the rat." *J. Biol. Chem.* **214**:463-473 (1955).
Schwarz, K., and C. M. Foltz. "Factor 3 activity of selenium compounds." *J. Biol. Chem.* **233**:245-251 (1958).
Schweizer, W. "Studies on the effect of l-tyrosine on the white rat." *J. Physiol.* **106**: 167-176 (1947).
Scott, E. B. "Histopathology of amino acid deficiencies. V. Isoleucine." *Proc. Soc. Exptl. Biol. Med.* **92**:134-140 (1956).
Scrimshaw, N. S., *et al.*, in *Amino Acid Malnutrition*. Rutgers Univ. Press, New Brunswick, N. J., 1957.
Scrimshaw, N. S., R. Bressani, M. Behar, and F. Viteri. "Supplementation of cereal proteins with amino acids I and II Effect of supplementation of corn-masa at (I) high levels, (II) intermediate levels of protein intake on the nitrogen retention of young children." *J. Nutrition.* **66**:485-514 (1958).
Sebrell, W. H., Jr., and E. G. McDaniel. "Amino acids in the production of blood constituents in rats." *J. Nutrition* **47**:477-486 (1952).
Sheft, Bernice B., and Helen Oldham. "Amino acid intakes and excretions during pregnancy." *J. Am. Dietet. Assoc.* **28**:313-318 (1952).
Singal, S. A., S. J. Hazan, V. P. Sydenstricker, and J. M. Littlejohn. "The production of fatty livers in rats on threonine- and lysine-deficient diets." *J. Biol. Chem.* **200**:867-874 (1953).
Singal, S. A., S. J. Hazan, V. P. Sydenstricker, and J. M. Littlejohn. "The lipotropic action of threonine and related substances in the rat." *J. Biol. Chem.* **200**:883-886 (1953).
Singal, S. A., V. P. Sydenstricker, and J. M. Littlejohn. "Further studies on the effect of some amino acids on growth and nicotinic acid storage of rats on a low casein diet." *J. Biol. Chem.* **176**:1063-1068 (1948).
Smuts, D. B. "The relation between the basal metabolism and the endogenous nitrogen metabolism, with particular reference to the estimation of the maintenance requirement of protein." *J. Nutrition* **9**:403-433 (1935).
212. Smuts, D. B., H. H. Mitchell, and T. S. Hamilton. "The relation between dietary cystine and the growth and cystine content of hair in the rat." *J. Biol. Chem.* **95**:283-295 (1932).
213. Snyderman, Selma E., and L. E. Holt, Jr. "Further observations on amino acid requirements of infants." (Abst.) *A.M.A.J. Diseases Children* **94**: November (1957).
214. Snyderman, Selma E., *et al.* "The phenylalanine requirement of the normal infant." *J. Nutrition* **56**:253-263 (1955).
215. Snyderman, Selma E., *et al.* "The essential amino acid requirements of infants: Lysine." (Abst.) *Federation Proc.* **15**:573 (1956).
216. Snyderman, Selma E., *et al.* "Valine." (Abst.) *Federation Proc.* **16**:399 (1957).
217. Snyderman, Selma E. and L. E. Holt., Jr. Personal communication.
218. Sorg-Matter, Hélène. "The quantitative law of the minimal nitrogen metabolism of the homeotherms." *Arch. Internat. Physiol.* **30**: 126-132 (1928).
219. Spies, T. D., C. Cooper, and M. A. Blankenhorn. "The use of nicotinic acid in the treatment of pellagra." *J. Am. Med. Assoc.* **110**: 622-627 (1938).
220. Sprunt, D. H., and C. C. Flanigan. "The effect of malnutrition on the susceptibility of the host to viral infection." *J. Exptl. Med.* **104**:687 (1956).
221. Stare, F. J. "Nutrition and aging." *J. Am. Geriatrics Soc.* **3**:767-771 (1955).
222. Steele, R. "The formation of amino acids from carbohydrate carbon in the mouse." *J. Biol. Chem.* **198**:237-244 (1952).
223. Sullivan, M. X., W. C. Hess, and W. H. Sebrell. "Studies on biochemistry of sulphur; preliminary studies on amino-acid toxicity and amino-acid balance." *U. S. Public Health Reports* **47**:75-83 (1932).
224. Sure, B. "Improvement in protein efficiency of whole wheat cereal breakfast food with amino acids lysine and threonine and vitamin B_{12}." *Arch. Pediat.* **69**:359-365 (1952).
225. Sure, B. "Influence of addition of certain amino acids and vitamin B_{12} to proteins in enriched milled wheat flour on growth, protein efficiency, and fat deposition." *J. Agr. Food Chem.* **5**:373-375 (1957).
226. Swanson, Pearl, Elizabeth Willis, and Pauline Mairs. "Do Iowa women eat enough protein?" *Iowa Farm Sci.* **9**(8):14 (1955).
227. Swendseid, Marian E., *et al.* "Amino acid requirements of young women based on nitrogen balance data. I. The sulfur-containing amino acids." *J. Nutrition* **58**:495-505 (1956).

228. *Ibid.*, "II. Studies on isoleucine and minimum amounts of the eight essential amino acids fed simultaneously," 507-517.
229. Sydenstricker, V. P., W. K. Hall, L. L. Bowles, and H. L. Schmidt, Jr. "The corneal vascularization resulting from deficiencies of amino acids in the rat." *J. Nutrition* **34**:481-490 (1947).
230. Terroine, E. F. "The protein component in the human diet." *Quart. Bull. Health Organization, League of Nations* **5**:427-492 (1936).
231. Thomas, K. "Ueber die biologische Wertigkeit der stickstoff-substanzen in verschiedenen Nahrungsmitteln." *Arch. Physiol.* 219-302 (1909).
232. Thompson, R. C., and J. E. Ballou. "Studies of metabolic turnover with tritium as a tracer. V. The predominantly nondynamic state of body constituents in the rat." *J. Biol. Chem.* **223**:795-809 (1956).
233. Trulson, M., D. M. Hegsted, and F. J. Stare. "New York State Nutrition Survey. I. A nutrition survey of public school children." *J. Am. Dietet. Assoc.* **25**: 595-605 (1949).
234. *Ibid.*, "II. A one-day study of food intake of adults," 669-676.
235. Tucker, Ruth E., *et al. Cooperative Nutritional Status Studies in the Northeast Region. IV. Dietary Findings.* Rhode Island Agr. Expt. Sta. Bull. 319, 1952, 24 pp.
236. Udupa, K. N., J. F. Woessner, and J. E. Dunphy. "The effect of methionine on the production of mucopolysaccharides and collagen in healing wounds of protein-depleted animals." *Surg., Gynecol. Obstet.* **102**:639-645 (1956).
237. Vohra, P., and F. H. Kratzer. "Graying of hair in rats fed on ration deficient in lysine." *Science* **124**:1145 (1956).
238. Wainio, W. W., *et al.* "Oxidative enzymes of the liver in protein depletion." *J. Nutrition* **49**:465-483 (1953).
239. Wainio, W. W., *et al.* "Enzymes in protein depletion. II. Oxidative enzymes of the heart ventricle." *J. Nutrition* **52**:565-573 (1954).
240. Waterlow, J. C., and N. S. Scrimshaw. "The concept of kwashiorkor from a public health point of view." *Bull. World Health Organization* **16**:458-464 (1957).
241. Waterlow, J. C., and Joan M. L. Stephen, eds. *Human Protein Requirements and their Fulfillment in Practice.* Proceedings of a conference at Princeton, 1955, sponsored jointly by FAO, WHO, and Josiah Macy, Jr., Foundation. New York, 1957. 193 pp.
242. Westerman, Beulah D., Bette Hays, and Be nice Schoneweis. "Improving the nutriti value of flour. VII. Supplementing the pr tein in flour with amino acids." *J. Nut tion* **61**:137-148 (1957).
243. Whipple, G. H., F. S. Robscheit-Robbins, ar L. L. Miller. "Blood protein regeneratic and interrelation." *Ann. N.Y. Acad. Sci.* 4 317-326 (1946).
244. Widdowson, E. M., and R. A. McCanc "Studies on the nutritive value of bread ar on the effect of variations in the extractic rate of flour on the growth of undernourishe children." *Med. Research Council* (Brit Spec. Rep. Ser. No. **287**:1-137 (1954).
245. Wiehl, Dorothy G., and W. T. Tompkin "Size of babies of obese mothers receivir nutrient supplements." *Milbank Memori Fund Quart.* **32**:125-140 (1954).
246. Wilcox, E. B., H. L. Gillum, and M. M Hard. *Cooperative Nutritional Status Studi in the Western Region. I. Nutrient Intak* Utah Agr. Expt. Sta. Bull. 383, July 195 44 pp.
247. Willcock, Edith G., and F. G. Hopkins. "Th importance of individual amino acids i metabolism; observations on the effect c adding tryptophane to a diet in which zein the sole nitrogenous constituent." *J. Physio* **35**:88-102 (1906).
248. Williams, C. D. "A nutritional disease c childhood associated with a maize diet. *Arch. Disease Childhood* **8**:423-433 (1933)
249. Williams, H. H., *et al.* "Estimation of growt requirements for amino acids by assay of th carcass." *J. Biol. Chem.* **208**:277-28 (1954).
250. Williams, J. N., Jr., W. J. Monson, A. E Harper, and C. A. Elvehjem. "Further studie of the relationship of vitamin B_{12} to enzyme in rat liver." *J. Biol. Chem.* **202**:607-61 (1953).
251. Woolley, D. W. *A Study of Antimetabolites* John Wiley & Sons, Inc., New York, 1952 269 pp.
252. Wretlind, K. A. J. "Effect of synthetic amino acids essential for growth on body weight o growing rats," *Acta Physiol. Scand.* 1 (Suppl. 59): 1-101 (1949); "Effect or growth and toxicity of the two isomers o methionine," *Ibid.*, **20**:1-12 (1950); "Availa bility for growth and toxicity of l- and d-phenylalanine," *Ibid.*, **25**:276-285 (1952)
253. Wretlind, K. A. J., and W. C. Rose. "Thia mine requirement for growth and utilizatio of its optical isomers." *J. Biol. Chem.* **187** 697-703 (1950).

The following publications on related subjects are available from the Printing and Publishing Office of the National Academy of Sciences—National Research Council.

Recommended Dietary Allowances. Food and Nutrition Board. Revised 1958; 36 p., paper; **$1.00. Pub. 589.** Table of amounts of nutrients recommended for maintenance of good nutritional status, with explanatory text.

The Role of Dietary Fat in Human Health. Food and Nutrition Board. 1958; 32 p., paper; **$1.00. Pub. 575.** Chemistry of food fats, fat metabolism (including digestion and absorption, lipid transport, and fat synthesis and utilization by body tissues), nutritional role of food fats, and relationships of cardiovascular disease and dietary fat. References.

Sodium-Restricted Diets. Food and Nutrition Board. 1954; 71 p., paper; **$1.00. Pub. 325.** Normal physiology of sodium metabolism, use of sodium-restricted diets in disease states, complications of sodium restriction, planning sodium-restricted diets, sources of sodium, analytical methods for sodium, salt substitutes. Tables, including sodium and potassium content of foods and sodium in public water supplies.

The Problem of Providing Optimum Fluoride Intake for Prevention of Dental Caries. Food and Nutrition Board. 1954; 15 p., paper; **$0.50. Pub. 294.** Conclusions are presented on the basis of studies of the magnitude of the caries problem, conventional approaches to prevention, the efficacy and safety of water and other possible vehicles.

The Composition of Milks, by Icie G. Macy, Harriet J. Kelly, and Ralph E. Sloan. Food and Nutrition Board. 1953; 70 p., paper; **$1.00. Pub. 254.** A compilation of the comparative composition and properties of human, cow, and goat milk, colostrum, and transitional milk, giving physical properties, fat characteristics, inorganic and organic chemical components, and vitamin content. Summary tables and bibliography.

Nutrition Surveys: Their Techniques and Values. Food and Nutrition Board. 1949; 144 p., paper; **$1.50. Bulletin 117.** The purpose and value of nutrition surveys; the interpretation of dietary surveys and of chemical tests; clinical diagnosis in nutrition surveys; and the relation of the survey to community nutrition programs, health departments, and schools of medicine and public health.

Bull. 123. Maternal Nutrition and Child Health, by Kirsten Toverud, Genevieve Stearns, and Icie G. Macy. Food and Nutrition Board. 1951; 174 p., paper; **$2.00.** A comprehensive survey of the present knowledge of the nutrition of the mother in relation to the health and vigor of the infant at all stages of his development, including sections on specific nutritional factors and on prematernal, prenatal, and postnatal care. Bibliography.

Cereal Enrichment in Perspective, 1958. Food and Nutrition Board. 1958; 20 p., paper, **free.** This publication covers pertinent literature from 1950 to the present and includes a brief review of the early history of enrichment. Its primary purpose is to analyze the present situation in order to foster interest in future promotion of enrichment of staple cereal foods such as bread, corn and macaroni products, and rice. It also covers enrichment policies in other countries; public health benefits and dietary trends in relation to enrichment.

THE FOOD AND NUTRITION BOARD

The Food and Nutrition Board, established in 1940 under the Division of Biology and Agriculture of the National Academy of Sciences—National Research Council, serves as an advisory body in the field of food and nutrition. It promotes needed research in the broad field and helps interpret nutritional science in the interests of the public welfare. The Board may act on its own initiative or on request from public or private agencies.

The members of the Board are appointed from among leaders in the sciences related to food and nutrition on the basis of their qualifications of experience and judgment to deal with the broad problems that come before the Board. Appropriate contact with Federal agencies, scientific societies, and other associations is maintained through liaison representatives appointed from their respective organizations. Specific activities of the Board are carried on by committees composed of experts in each field. Members of the Board and its committees serve without compensation beyond their actual expenses.

Financial support for publications and meetings of the Board is primarily provided by foundations such as the Milbank Memorial Fund, the Nutrition Foundation, and the Williams-Waterman Fund of the Research Corporation. Funds for specific purposes may come through government contract or grants from industry.

NATIONAL ACADEMY OF SCIENCES—NATIONAL RESEARCH COUNCIL

The National Academy of Sciences—National Research Council is a private, nonprofit organization of scientists, dedicated to the furtherance of science and to its use for the general welfare. The Academy itself was established in 1863 by the terms of a Congressional charter under which it is empowered to provide for all activities appropriate to academies of science and is required to act as an adviser to the Federal Government in scientific matters. The National Research Council was established by the Academy in 1916, at the request of the President of the United States, to enable scientists generally to associate their efforts with those of the limited membership of the Academy. With funds contributed from both public and private sources, the Academy and its Research Council work to stimulate research and its applications, to survey the broad possibilities of science, to promote effective utilization of the scientific and technical resources of the country, to serve the Government, and to further the general interests of science.

Dn/24-8-89

Mh. 9.9.89

C. F. T. R. I. LIBRARY, MYSORE.

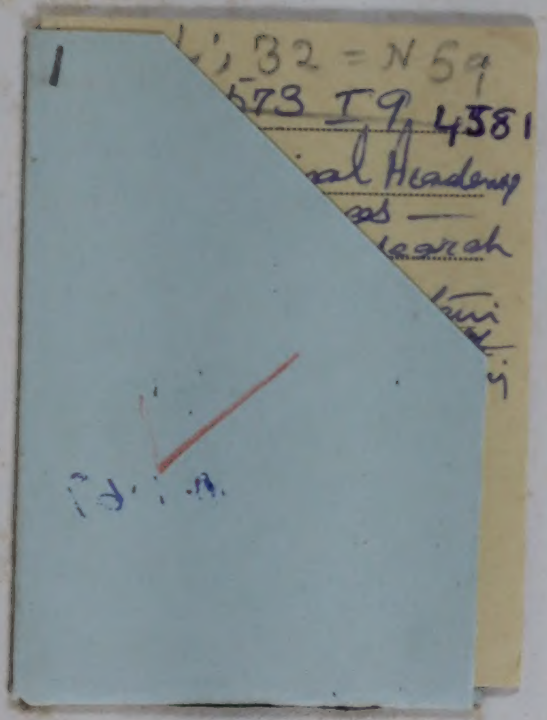
CHECKED
5-5-97

18-4-95 6.4.95

CHECKED
2008

VERIFIED
2013

CFTRI-MYSORE
4381
Evaluation of pr...


1
L; 32 = N 59
573 I9 4581